

THE AMERICAN JOURNAL
OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

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NUMBER 1

THE PATHOLOGY OF BILHARZIASIS *

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BILHARZIA; OBSERVATIONS ON ITS PATHOLOGY

Egyptian hematuria has been known from the very earliest times. Bilharz, working in Egypt in 1831, discovered a trematode worm in the mesenteric vessels of men who suffered from this disease and gave us the first accurate description of the parasites. In 1894, Looss¹⁰ described the parasites more fully and outlined the life cycle in the human body. The mode of transfer from patient to patient was not fully investigated until Leiper,⁹ working in the bilharzia mission in Egypt in 1915, discovered that in common with other parasitic trematodes, the bilharzia parasite is transmitted from host to host through an intermediate host. In this case the intermediate hosts are small fresh water snails of the genus *bullinus* and genus *planorbis*.

There are three closely related trematodes of the genus *Schistosoma* that are now known to produce characteristic lesions in man. *Schistosoma japonicum* occurs in the far East. The other two, *Schistosoma mansoni* and *Schistosoma hematobium* occur in Egypt and along the eastern coast of Africa to the Cape. The disease is found all along northern Africa and in the Congo, but most commonly in Egypt. It occurs in the West Indies and in Central America. Throughout the rest of the world occasional cases occur, but they are usually imported from some area where the disease is endemic. The disease is limited in its distribution by the distribution of the intermediate host and the habits of the people with regard to exposure to water that is contaminated.

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Conditions in the Nile valley, particularly in the delta region, are peculiarly favorable to the distribution and propagation of the bilharzia parasite. The congestion of population and primitive habits of the people insure gross contamination of the water; the tropical climate and perennial irrigation insure a wide distribution of the intermediate hosts of the parasite, and the agricultural habits of the people insure the adequate exposure of the skin to water containing infective forms of the parasite. For these reasons the incidence of infestation in a land like Egypt is very high, particularly among the peasantry.

Ferguson⁷ in 1913 stated that in more than 600 postmortems on male subjects, performed in the medical school in Egypt, no less than 61 per cent of the males between 5 and 40 years of age were infested. Madden¹² in 1910 said that 10 per cent of the total mortality in Egypt was due to bilharzia. He points out the fact that his figures are based on the number of deaths occurring in the hospital, and as very many ill patients go home to die, and are therefore not included in the record, the percentage is undoubtedly much higher. Of 3485 native patients treated in the American Hospital at Tanta since 1924, 35.3 per cent were treated for bilharzia and its complications. The incidence of the disease among the peasantry of the country is much higher than these figures indicate, for there are patients with mild infection who complain of no symptoms and yet occasionally pass blood and ova in the urine and feces.

The American Mission Hospital is situated at almost the geographic center of the delta of the Nile and affords an ideal opportunity to see a large amount of bilharzial material both clinically and in the laboratory. Approximately 1200 native patients are housed for periods varying from one to three weeks during each year. A large number of these patients come suffering from advanced bilharziasis. Surgical intervention is often necessary for the relief of complications arising from the infestation. The material from which this study was made was collected over a period of five years residence in Tanta. All specimens removed surgically were examined and the more interesting of them preserved in 10 per cent formalin and brought to America for further study. They were cut, embedded in paraffin, sectioned, and stained with hematoxylin and eosin in the laboratory of the City Hospital in Cleveland.

All of the specimens were from native Egyptians and contained

bilharzia parasites. Many of them were polypi removed from the rectum and bladder. In many cases of inguinal hernia, the peritoneal sac was found to be infiltrated with ova. Where this was the case the sacs were kept for study. In the course of laparotomies for bilharzial tumors, specimens were removed from the abdomen. These specimens consisted of markedly involved epiploic appendages, pieces of omentum and peritoneum containing nodules of ova. In cases where the vermiform appendix was removed from native patients, it was always examined for bilharzial and amebic infections. Interesting specimens showing bilharzia in the female genital tract were obtained during the course of gynecological operations for other than bilharzial conditions. The effects of oviposition under the squamous epithelium of the vagina were studied in sections obtained from cases of marked vaginal polyposis by means of the curette. Sections from the margins of perineal fistulae near the anus and about the scrotum were used to study the effects of ovaposition under the true skin. Four partial autopsies on patients who died in the hospital furnished the rest of the material.

The study is based, specifically, on a series of sixty-five specimens that show the intestinal mucosa; fourteen specimens of tissue including the peritoneum; twelve vermiform appendices, and forty other tissues from various parts of the body. All of the tissues in this series showed bilharzial involvement.

In order to understand the pathology of bilharzia, it is necessary to know the life history of the parasite that causes it. In general the two species known in Egypt are very similar and one description will, for our purpose, suffice for both. A more detailed description is found in "The Practice of Medicine in the Tropics" by Byam and Archibald.

THE LIFE HISTORY OF THE PARASITE

The animal is a bisexual trematode. The male is a white to gray worm, 1 to 1.5 cm. long. The anterior portion of the body is cylindrical and is armed with an anterior and ventral sucker. The portion of the body posterior to the ventral sucker is leaf-like when spread out and terminates in a rounded blunt extremity. In the natural condition this flattened posterior portion of the body is rolled in from the two sides, forming a longitudinal ventral groove, the gynecoproic canal. The result is a rounded appearance. (Figs. 1 and 5.)

The female parasite is filamentous, cylindrical in shape and much longer than the male. Two suckers, similar to those described for the male, are seen toward the anterior end of the body. In nature the female is usually partially enveloped by the male and lies in the gynecapric canal (Figs. 2, 3, 4). Both the male and the female parasite have a very primitive alimentary canal beginning at a stoma in the anterior end of the body and ending blindly posteriorly. The anatomy of the generative organs differs somewhat in the different species, but in general is similar. The ovary is located in the posterior half of the female worm and the oviduct passes forward to join the vitelline duct. The uterus extends anteriorly to the genital pore, just posterior to the ventral sucker.

The ova measure about 0.15 by 0.06 mm. and are just about the lower limits of visibility to the naked eye. Those of the species *hematobium* have a terminal spine (Fig. 13), and those of the genus *mansoni* have a lateral spine (Fig. 8).

The parasites attain their adult form in the liver and vessels of the portal system. When mature they migrate against the blood stream till they reach a capillary bed. Selection is apparent in that *S. mansoni* migrate to the capillary bed under the mucosa of the colon and rectum, and *S. hematobium* to the mucosa of the urogenital tract, particularly of the bladder.

In Tanta, Egypt, where these observations were made, double infections are common, and *S. hematobium* ova (terminal spined) are commonly found in the feces associated with the ova of *S. mansoni* (lateral spined). Dew,³ has pointed out the ease with which females of *S. hematobium* can migrate through the veins of the pelvic plexus to the region of the lower rectum and anus, in explanation of this fact. Fairley,⁴ has pointed out the frequency with which *S. hematobium* ova occur in the feces of patients who are not infected with *S. mansoni*. *S. mansoni* ova occur in the urine, but only in heavy infestations and much less commonly than in the feces. In the year 1926 a record was kept of the routine feces and urine examinations that were made in Tanta Hospital. Many of these were single examinations and therefore the results cannot be taken as indicating the total incidence of infestation. The results are interesting in that they give an index to the relative frequency with which the two types of parasite occur in the branches of the pelvic venous plexus and the inferior mesenteric veins (Table I).

In the study of tissues the selective deposition of ova is still more clearly evident. Both in cut sections and in tissues digested by 5 per cent alkali the general region from which the tissues come can be determined by the character of the ova present. In tissues from the bladder, lower third of the ureters, urethra and genitalia, practically all of the ova in the cases studied in this series were terminal

TABLE I

Examinations Showing Distribution of Ova in Urine and Feces

Number of Feces Examinations 860		Number of Urine Examinations 888	
Positive for <i>S. mansoni</i>	Positive for <i>S. hematobium</i>	Positive for <i>S. mansoni</i>	Positive for <i>S. hematobium</i>
267 or 31 %	31 or 3.6 %	20 or 2.2 %	302 or 22.8 %

spined. In tissues from the rectum, around the anus and in the vagina, both lateral and terminal spined ova were found, the former predominating. In the mucosa of the colon, in the parietal and visceral peritoneum, retroperitoneal lymph nodes, spleen and liver, lateral spined ova greatly out-numbered the ova of *S. hematobium* and in many cases the latter were not found.

In oviposition the female parasite forces the anterior end of her body into the smaller capillaries as far as she can and then deposits her ova. The ova are left in the dilated capillary space as the female withdraws. The elastic vessel walls close down on the ovum, forcing its sharp spine against the wall. The abrasion and a peculiar irritating quality that the ovum seems to possess cause a severe inflammatory reaction, swelling of the tissue, and finally ulceration of the epithelial covering, allowing the ovum to escape into the bladder or gut as the case may be. In a land like Egypt the ovum then readily passes into the water of the canals and streams.

The ovum itself is innocent of any ability to infest a new human host either by contact or by ingestion. If allowed to dry, it quickly perishes, but in fresh water it hatches almost at once into a miracidium. This is a unicellular ciliated form in some ways resembling a paramecium. It swims around rapidly in water but quickly perishes if it does not find a suitable host. Such hosts abound in the waters of Egypt. The fresh water snails of the genus *bullinus* and genus

planorbis are readily attacked and infected. Four to five weeks after infestation of the snail there emerge from its body cercariae. These are small tadpole-like forms that are just about visible to the naked eye and can be seen easily with the hand lens. This form swims about in the water for from twenty-four to thirty-six hours. Before the end of this time they must find a new human host or they perish. Contact of water harboring this form of the parasite with any surface of the body or the mucous membranes of the mouth and throat leads to infestation. The cercariae attach themselves to the skin or mucous membrane by means of their suckers, lose their tails, and penetrate to the subcutaneous lymphatics. They then start on a course of migration in the body and in about six weeks the adult forms may be found in the liver and portal veins, completing the life cycle (Leiper⁹).

It is not known just how long the parasites continue to live and produce eggs in the tissues of the host. It is evidently quite a long period, perhaps years. It is certain that individuals who have long been away from sources of infestation in countries where the disease does not exist still pass living ova in the urine and feces. In one case, known to the author, a young Egyptian student who contracted the disease when a boy continued to pass living ova during the years of his study of medicine in America and was still passing them when he returned to Egypt.

CLINICAL MANIFESTATIONS

When the initial infection occurs there is a definite irritation of the skin due to the passage of the parasites. This is perhaps more marked in Japan than Egypt. At any rate, it has been described by Japanese writers who study the skin lesions in those who work in the rice paddies. In Egypt, skin lesions are seldom noticed and the patient does not seek medical aid because of them. As a rule, too, the infestation is so gradually acquired that the skin lesions are neither very widespread nor extensive at any one time. As the parasite penetrates into the deeper tissues, a small inflammatory nodule may be felt for a short time. Leiper describes an inflammatory skin lesion occurring in experimental animals exposed to bilharzial cercariae.

If only a few parasites have found an entrance to the body in a given exposure, the invasion is not attended by subjective symptoms.

If a massive invasion occurs there will be fever and malaise (Fairley⁴). In one case in our experience where a patient had been kept away from his home environment until he was free from infestation, and then allowed to return to his village, he went to work irrigating the fields and was exposed to heavy bilharzia infestation. A few weeks later he began to suffer from a severe typhoid-like fever with jaundice and marked prostration. Enteric fevers were ruled out by serologic tests and on clinical grounds. As he gradually recovered from his febrile attack, he developed symptoms of severe generalized abdominal bilharziasis with both types of ova in the urine and feces. A similar febrile course during invasion by the parasites has been described in foreign soldiers who have bathed in the canals of Egypt (Fairley⁴), and in sailors who, while on leave, bathed in fresh water lakes in China (Laning⁸).

In most cases the infestation of the individual begins in early childhood and continues throughout life. The peasantry are constantly exposed and constantly adding to their parasitization. If the number of parasites is not excessive, the host is able to bear his infestation fairly well, but when the infestation is excessive or complications set in, he succumbs.

PATHOLOGY OF BILHARZIA

The early lesions of bilharzia are due to the presence of the parasites and their ova in the tissues. The later lesions are due to disturbances of structure and function arising from the efforts of the body to throw off the infection and to repair the damage. Later lesions are also the result of bacterial invasion of devitalized tissues. The first clinical evidence of oviposition is passage of blood, which is caused by an inflammatory reaction in the mucosa either about the trigone in the bladder, or in the rectum, or in both. Soon there develop small areas of a sandy nature surrounded by hyperemia, or still more frequently the whole mucosa loses its normal bright appearance and becomes sandy (Fairley⁴ and Madden¹¹). The sandy appearance is due to clumps of ova lying in the tunica propria beneath the mucosa. The deposit of ova may extend until the mucosa looks like very fine sandpaper. In the bladder and ureters, urine salts are sometimes deposited on such a surface, giving rise to the foundation for a stone. Mucous membranes thus filled with ova are easily abraded and bleed readily. In order for the ova to escape

from the tissues, the mucosa must be broken through. Our studies show that this is accomplished by a process of ulceration. An intense inflammatory reaction occurs with the accumulation of many leucocytes, lymphocytes and endothelial cells. Among the leucocytes is a large number of eosinophiles. Irritation of the mucosa and the loss of its normal resistance to bacterial invasion result in actual abscess formation. The abscesses are as a rule very superficial. As they rupture into the lumen of the intestine or bladder, they discharge ova together with their contents. Granulations spring up at once in the ulcerated areas and may become excessive. As they grow they continue to carry ova up through the mucosa to the surface. From the fact that adult parasites are commonly found in the loose areolar tissue under such areas of ulceration and granulation tissue, and from the large numbers of ova in such a place, it seems likely that the gravid female is attracted to areas where the processes of tissue repair are active. The presence of bilharzia parasites and their ova seems to stimulate the tissue to activity.

The softer the mucous surface and the more glandular its epithelium, the easier it is for ova to pass through it. The columnar epithelium of the colon affords the easiest access to the outer world and ova are discharged in very large numbers through lesions in its surface. There is always excessive production of mucus, and in many areas the intestinal epithelium shows mucinous degeneration (Fig. 14).

The ova are aided in passing through the bladder mucosa by the distension and contraction of the organ, and by superficial ulceration. Abscess formation, such as is seen in the rectum, has not been noted in the bladder sections studied. In the cervical and vaginal mucosa it is more difficult for ova to escape from the tunica propria into the vagina. In our specimens small abscesses were seen to have formed around groups of ova. Small fistulae allowed the contents to escape into the vagina. Polyps are formed in the mucous membranes of the colon and the bladder. Their formation depends on the degree of infestation and on peculiarities in the reactivity of the host. They vary in size, and are often so large and numerous as to cause marked obstruction of the bowel. Specimens 3 to 4 cm. in diameter were commonly seen. The polyp may consist of a single lobulê or it may be multilobular. The pedicle of the polyp may be very narrow and consist mostly of mucous membrane, in which case it is very friable;

or it may be broad and involve the deeper coats of the intestinal wall, in which case the polyp is likely to be very firm and fibrous. The lobules are covered with mucous membrane except for areas of ulceration where there is an abundant formation of granulation tissue.

In the Tanta cases polyps were found anywhere in the large intestine from the cecum to the anus, but were most often found in the descending colon, sigmoid and rectum. They occurred in a localized area or sometimes there was a diffuse polyposis of the whole intestine. In the bladder, polyps occurred less frequently than in the colon. They were located on the posterior wall near the trigone and were often associated with a malignant change. Very heavy infiltrations of the mucous membranes of the bladder and intestine sometimes occurred and resulted in a general thickening of their walls without the formation of polyps. In such a case the bladder had become a thick-walled non-contractile viscus with limited capacity and very septic walls. The sepsis is accentuated by the decomposition of blood and pus in the residual urine and a fatal issue is almost a certainty.

Microscopically the rectal polyp is seen to consist of a swollen, hyperplastic epithelium covering an irregular mass of connective tissue stroma. The epithelium is often broken by areas of granulation tissue and areas of mucinous degeneration of the goblet cells in the mucosa (Fig. 14). The stroma of the polyp is filled with bilharzia ova and in the vessels at its base there often are adult parasites of both sexes. If the polyp has been recently formed and is soft, a large number of polymorphonuclear cells, eosinophiles and lymphocytes are found in its stroma, and the ova appear undegenerated and intact (Fig. 6). If the polyp is older it is firmer, with a large amount of fibrous tissue in the stroma infiltrated chiefly with endothelial cells and lymphocytes (Fig. 11), and many of the ova are degenerate, the embryo is lost, and only the chitinous wall remains. The chitinous walls of ova are often surrounded by giant cells of the foreign body type and by pseudotubercles (Figs. 7 and 9).

Ova are much less frequently deposited under squamous epithelium than under either columnar or transitional epithelium. On the cervix, in the vagina and about the anus, however, such deposits are not uncommon. The ova are found in the corium and layers of the superficial fascia and are surrounded by an inflammatory reaction

and hyperplasia of the squamous cells. The result is a marked thickening and wrinkling of the surface. The papillae are hypertrophic and extend deeply into the corium. The epithelial thickening grossly resembles widespread confluent verrucae. Sections show the microscopic structure to be not unlike that of verrucae. As the thickening progresses, the tissues lose their vitality and small abscesses form about groups of ova in the corium. This is undoubtedly brought about by invasion of pus-forming bacteria. Abscesses form and the ulceration allows the offending ova to be discharged. The invaded area becomes very thick and edematous, with small and larger abscesses forming and rupturing on the surface. There is considerable production of granulation tissue and later of scar tissue. The epithelium becomes excessively thickened and rugose. Such a process, after it has continued for a long time, obliterates the normal structures. Cases are frequently seen in Egypt where the posterior and anterior culs-de-sac of the vagina are obliterated and the cervix is buried in a mass of scar tissue. The vagina itself may become constricted, causing more or less complete atresia.

Still less frequently ova are found deposited under the true skin. This occurs in the region of sinuses and other chronic inflammatory processes near the anus and about the genital orifices. Here, as under the moist squamous surfaces, there is no escape for the ova except through ulceration. Ova are not found in the epithelium but are seen closely related to the epithelial cells in an area of granulation or degeneration.

In all heavy infestations, ova are deposited in the muscular and serous coats as well as in the submucosa. Here the ova are in an abnormal situation and cannot escape from the tissues, so their life cycle cannot be completed. They are held prisoners, so to speak, in the tissues. The result is similar to that seen when other foreign bodies are deposited in the tissues. There is first an inflammatory reaction that is not suppurative. Large numbers of polymorphonuclear cells, lymphocytes and endothelial cells appear about the ova (Figs. 6, 8). Then, as the ova die, giant cells are seen (Figs. 7, 9). At times leucocytes and giant cells may be seen surrounding or actually within the ovum. Probably the leucocytes play a part in the removal of the embryo. The more resistant walls of the ovum remain unchanged or become calcified. Endothelial cells and lymphocytes become grouped about them and finally they are walled

off by dense fibrous tissue (Figs. 10, 11). These are the pseudotubercles very commonly seen in bilharzia tissue.

Grossly the tissue becomes hard and fibrous, cuts with a gritty feel, and presents a sandy appearance.

The peritoneum may be thickly studded with tubercles, usually distributed most abundantly throughout the lower abdomen and over the colon. These hard nodules varied in size in the specimens studied from those that could be scarcely seen by the naked eye to masses of tumor-like structure the size of a fetal head. The large masses involved all the walls of the intestine and the surrounding tissues, all being matted together in an inseparable mass. Some sections of severely involved intestines looked grossly like tumors, but microscopically showed only massive infiltration with ova, pseudotubercle formation, and excessive formation of fibrous tissue. The pseudotubercles did not show caseous necrosis.

In the interlobular veins of the liver the presence of the parasites and possibly products liberated by them causes, first, a marked enlargement, and later a peculiar periportal fibrosis, described as a pipe-stem cirrhosis (Symmers¹³). In young children and young adults, the liver extends down to the umbilicus and fills the whole upper right quadrant of the abdomen. In later life and in older patients the liver is smaller and nodular. Grossly it is coarsely lobular and very firm. It cuts with very much increased resistance, and throughout its substance are very marked bands and cords of fibrous tissue about the portal spaces. Dew³ describes all grades of tissue change in the liver from a mild generalized fibrosis with hepatic enlargement such as is seen in earlier and milder cases and especially in connection with *S. hematobium* infections, to the severe periportal cirrhosis seen in later severe infections with *S. mansoni*. The specimens examined in this study were of the severe type and the periportal cirrhosis was very marked. All of these cases were heavily infested with *S. mansoni* parasites and showed pronounced intestinal involvement.

Microscopically the portal spaces showed a decided proliferation and tortuosity of the bile ducts. The veins were dilated and contained parasites. There was a great increase of the fibrous tissue which contained large numbers of lateral spined ova. In one case examined, the cords of fibrous tissue surrounding the portal spaces were two to three centimeters in diameter. Contraction of these

bands had resulted in compression of the liver parenchyma and reduction in the size of the organ. The liver cells were atrophic, due to compression, and endothelial cells contained a brown pigment.

Splenic enlargement is commonly, but not constantly, associated with bilharzia infection. It is apparently most common in *S. mansoni* infections. The average weight of the spleen in five cases at Tanta where the spleen was removed surgically was 1168 gm. The organs were firm and uniform in consistence, cut with increased resistance, and presented a pulp that did not scrape away readily. Microscopically there was evident increase in the amount of fibrous tissue. The follicles and trabeculae were widely separated and the splenic pulp was very cellular and fibrous. No parasites or ova were seen. The cells were mainly of the endothelial type and contained a brown pigment that was similar in nature to that found in the enteric canal of the bilharzia parasite (Manson-Bahr¹), and in the endothelial cells of the liver.

In severe infestations ova are found deposited in all abdominal organs. In this study they have been found in the walls of the small intestine, appendix, cecum and colon; in the pancreas, liver, retroperitoneal lymph nodes and omentum; in all points of the peritoneum, particularly over the colon, bladder, and in the sacs of inguinal herniae; in the ureters, bladder, seminal vesicles, epididymis, testes, ovary, uterus, cervix and vagina. Ova have been discovered in the lungs, brain and kidneys.

The later complications of bilharziasis are those that often cause the greatest debility and bring about a fatal issue. In itself bilharzia is not likely to be a lethal disease. The most frequent complications occur in the urinary system. One of the early manifestations of bilharzia infection is swelling about the ureteral openings into the bladder. Stasis in the ureters and kidneys follows. Bleeding into the bladder and ulceration of its mucosa sooner or later results in cystitis. Long-continued irritation of the bladder wall causes thickening and loss of elasticity. Residual urine and blood in the bladder result in still further sepsis. Ascending pyelitis and pyonephrosis are very common sequels. Deposits of urine salts occur and stones are formed in kidney, ureter and bladder.

Inflammation about the trigone and urethra causes swelling and later strictures which interfere with emptying the bladder. Abscesses form about the trigone and in the prostate. Extravasation

of urine occurs and abscesses and later sinuses form in the perineum and scrotum. Frequently cases were seen where extravasation caused the scrotum to slough away, leaving the testes exposed. In one case the extravasation extended up in the abdominal wall as high as the umbilicus, resulting in severe sepsis and death.

About the anus and rectum, abscesses and sinuses are common. Stricture of the rectum is frequent as the result of trauma to the walls of the gut. Polyps in the intestines cause the patient to go to stool constantly, and the straining results in relaxation of the perineal muscles, edema of the gut and prolapse of the intestines. As much as eight inches of the lower bowel have been seen protruding from a patient after he has been at stool. One of the specimens studied was from such a case. Constant straining during his illness had caused marked prolapse of the lower bowel. A conical mass of swollen, ulcerated, everted intestine protruded from the anus, which was much dilated. After death the bowel was removed. The lumen was found to contain bilharzial polyps. The mucosa was very much inflamed, in areas ulcerated, and contained large numbers of ova. The walls of the intestine and the surrounding fascial supports were edematous and contained bilharzia ova and an inflammatory exudate.

Ileus is common, due to adhesions about the intestine or to actual narrowing of its lumen by fibrosis or by polyps.

Abscess formation in the liver or abdomen has not been noted. Bilharzial appendices sometimes become septic and are difficult to treat surgically because of the low resistance of the surrounding tissue.

Thrombosis of the veins is common. It may occur in the mesenteric veins and cause an ileus and gangrene of the gut. Cases were seen in which the femoral vessels, abdominal vena cava and portal vessels were involved. In one specimen studied there was extensive mesenteric thrombosis and thrombosis of all branches of the portal veins in the liver. The vessels contained large numbers of parasites.

In our experience the blood in bilharziasis shows anemia that is out of proportion to the blood losses from the bladder and bowel. In cases of average severity the hemoglobin ranges between 30 per cent and 45 per cent, the red count from less than one million to three million. The white count is usually about 10,000 to 12,000 and there is a marked eosinophilia. Day,² in his report on the blood

changes in bilharziasis, selected early and uncomplicated cases and concluded that bilharziasis *per se* produces only slight anemia, and the most characteristic change is eosinophilia.

Tumors are commonly seen associated with bilharzial tissues. Ferguson,⁶ and Madden,¹¹ call attention to the tumors of the bladder and say that both sarcoma and carcinoma occur. True carcinoma of the bladder has frequently been seen during this study and in at least two instances tumors that look very much like sarcomas were found in the bladder.

In the colon and rectum two examples of mucinous carcinoma were seen, one in the appendix and the other in the rectum.

DISCUSSION

The study of this material was undertaken in the hope that the character of the pathologic processes resulting from the presence of bilharzia parasites and their ova in the tissues might be more clearly understood. The following points have been more clearly brought out.

1. It has been recognized that the presence of the parasites and their ova in the tissues is attended by a primary inflammatory reaction of a nonsuppurative nature. We wish to point out that whether this inflammation is followed by ulceration or fibrosis depends on the location of the deposition of ova. We believe that there is no inherent quality in the parasites or the ova that is sufficient, of itself, to produce ulceration. The ulcerative process seems to be dependent on the action of secondary bacterial invaders on tissues of diminished vitality. Where the tissues are not exposed to such invasion as, for example, in the peritoneum, omentum and deep tissues like muscle, abscess formation and the discharge of the ova do not commonly occur. Where they do occur, the process begins with suppuration in more superficial tissues. In superficial tissues near the epithelial lining of a hollow viscus or even under the skin, the primary inflammation is followed by ulceration and discharge of the ova.

In the deeper tissues and in the abdominal organs such as the liver, abscess formation has not been seen. The ova, at first surrounded by inflammatory cells, become isolated by fibroblasts and giant cells of the foreign body type. The ultimate result is the formation of a granuloma, the characteristic unit of which is the bil-

harzial pseudotubercle. The excessive fibrosis often resulting late in such a process produces marked distortion or even obliteration of structures, interferes with physiologic function and is the basis of many of the serious complications occurring late in the disease.

2. That the bilharzia parasite produces nocuous substances in the body of its host is evident on the basis of clinical experience with patients who have acquired a severe infection in a short period of time, and from experimental evidence (Fairley⁵), from animals infested with large numbers of cercariae. Experimental animals heavily infested die before oviposition occurs.

The nature of this nocuous substance is not clearly established but the following facts are noted in evidence that it is the result of simple protein splitting rather than toxin production:

(a) Histologically there is abundant evidence of the digestion and removal of the embryos by the phagocytic cells of the body. This is attended by blood changes that are characterized by a mild to severe anemia of the secondary type, slight leucocytosis and a marked relative increase in the number of eosinophiles and large mononuclear cells (personal observation, and Day²).

(b) Much pigment similar to that deposited in malarial infections is deposited in the internal organs, particularly the spleen and liver. This pigment is similar in character to that seen in the enteric canal of the parasites.

(c) Fairley has shown that there is a specific reaction in the body that results in a constant (88 per cent) fixation of complement when extracts of the livers of infected snails are used as the antigen.

(d) Clinical experience does not indicate the formation of immunity in the patient even after he has been long infected.

Grateful acknowledgment is made of the help and advice given by Professor H. T. Karsner and Professor Harry Goldblatt of Western Reserve University; and Doctor Otto Saphir of the Cleveland City Hospital. The photomicrographs were made by Professor Karsner.

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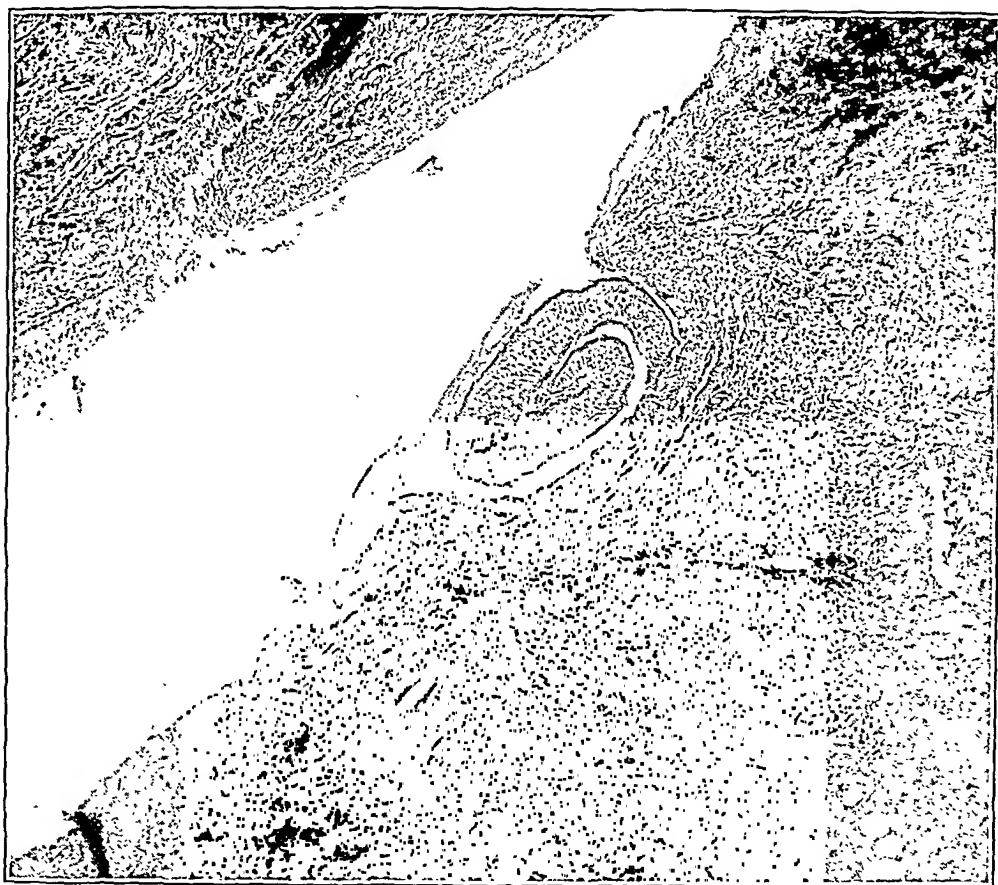
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DESCRIPTION OF PLATES

PLATE I

- FIG. 1. *Bilharzia* parasite, male, in an interlobular vein of the liver. Note the large size of the vessel and the surrounding fibrous tissue.
- FIG. 2. *Bilharzia* parasites, male and female, in the veins at the base of a rectal polyp. The male enfolds the female.



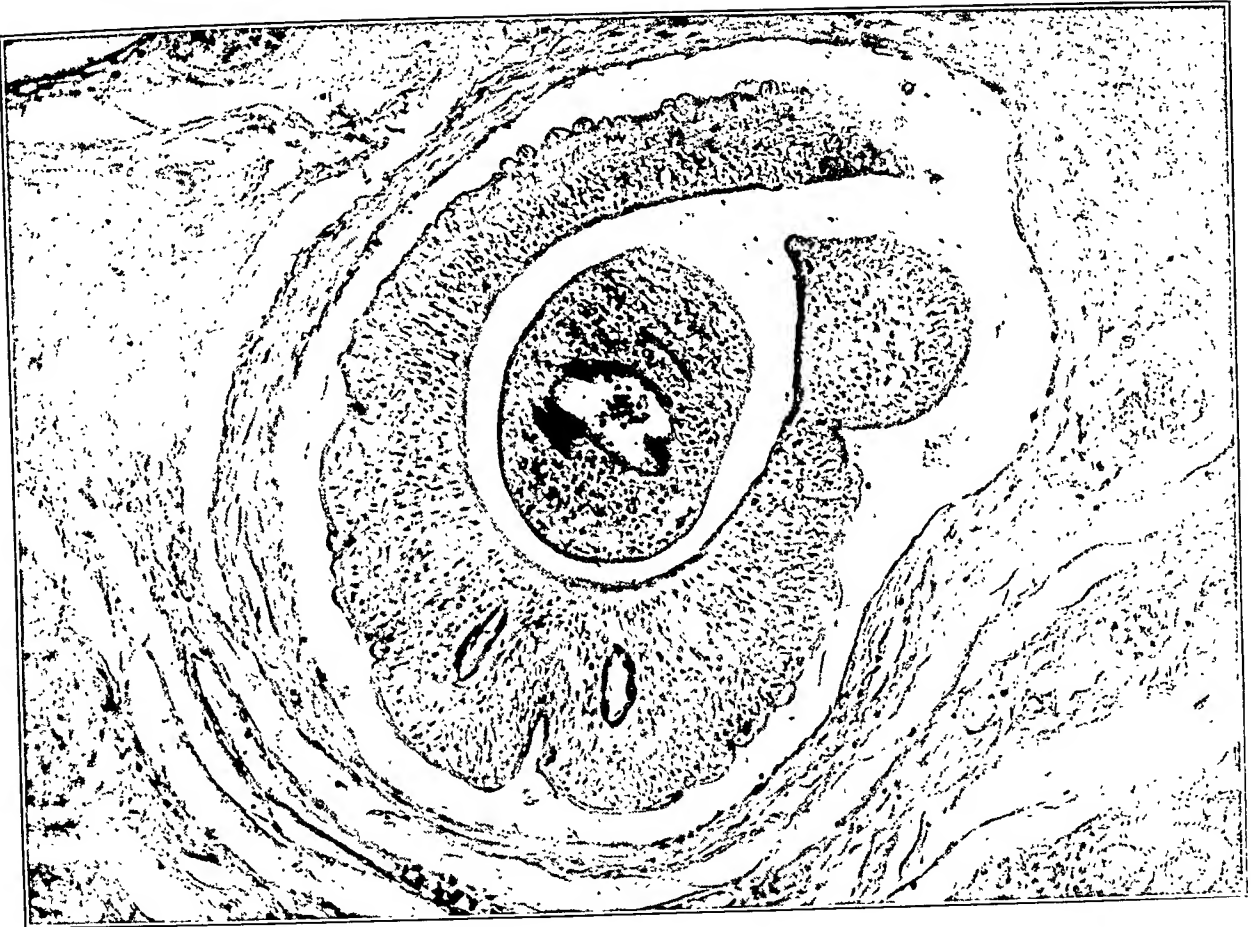
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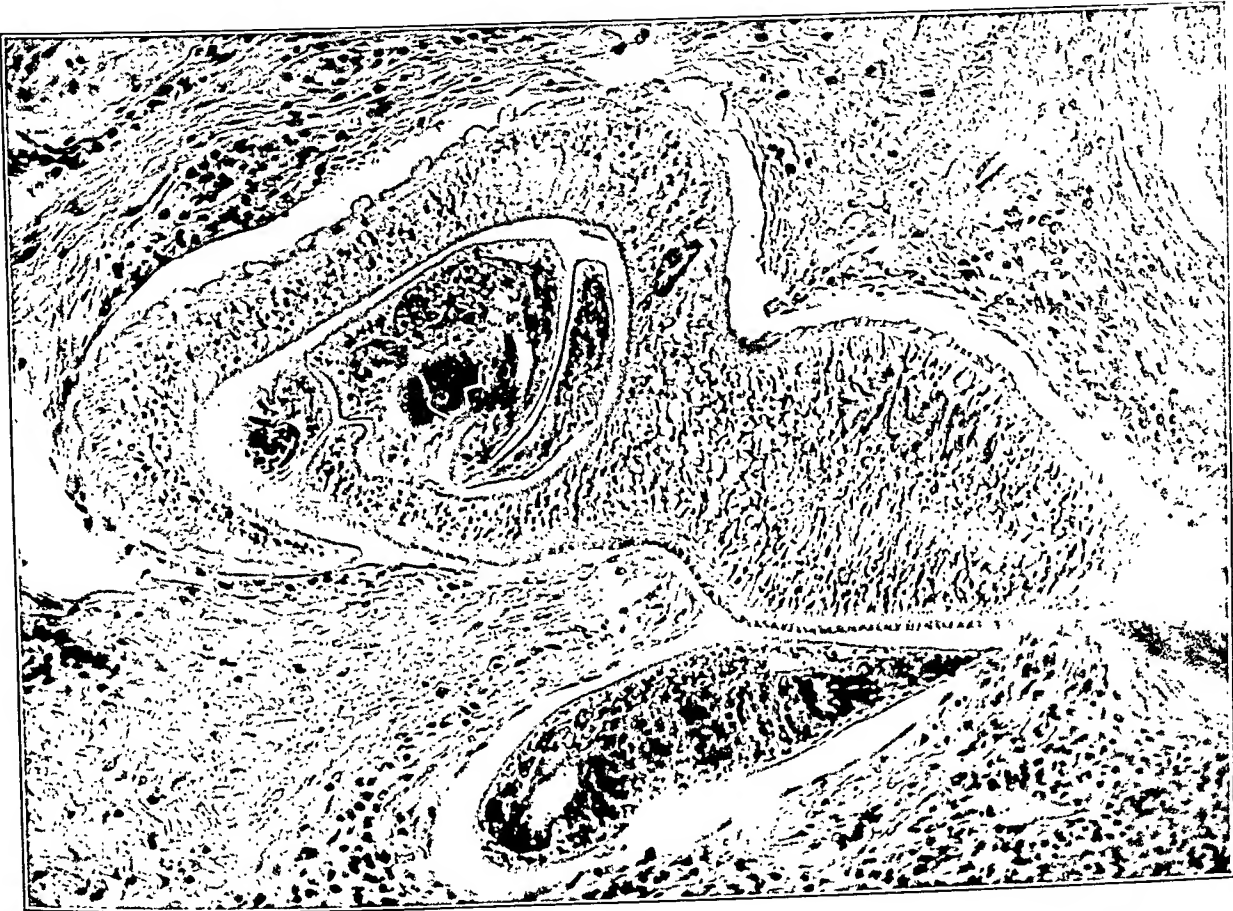
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PLATE 2

- FIG. 3. Bilharzia parasites, male and female; the cross-section taken posterior to the bifurcation of the intestinal canal of the male.
- FIG. 4. Bilharzia parasites, male and female, in cross-section through the ovary of the female.



3



4

PLATE 3

- FIG. 5. Bilharzia parasites, male and female. The females are not in the gynecapric canal of the male.
- FIG. 6. Bilharzia ovum, *S. mansoni*, from a rectal polyp, showing the surrounding inflammatory reaction. Many of the polymorphonuclear cells are eosinophiles.
- FIG. 7. Bilharzia ovum, *S. mansoni*, in a rectal polyp. This ovum is dead and a giant cell has formed against its shell and within its lumen.

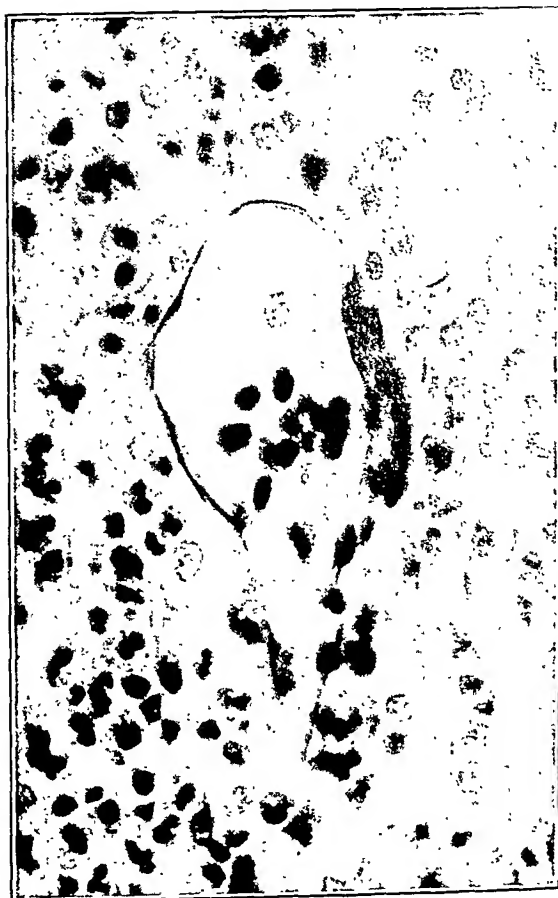


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Hutchison



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Pathology of Bilharziasis

PLATE 4

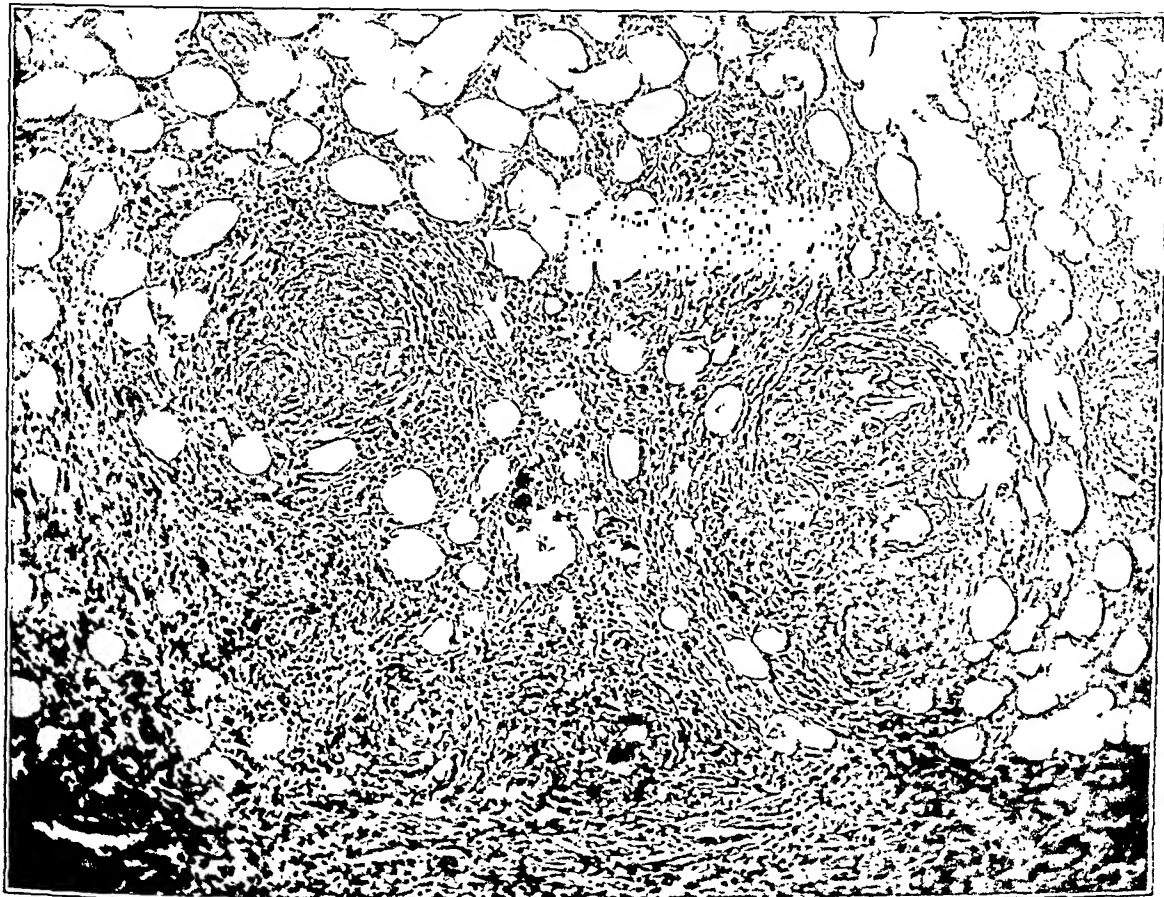
- FIG. 8. Bilharzia ovum, *S. mansoni*, showing the lateral spine. The embryo is replaced by tissue cells.
- FIG. 9. A very large giant cell about an empty ovum.
- FIG. 10. Pseudotubercles about ova in the fat of an epiploic appendage.



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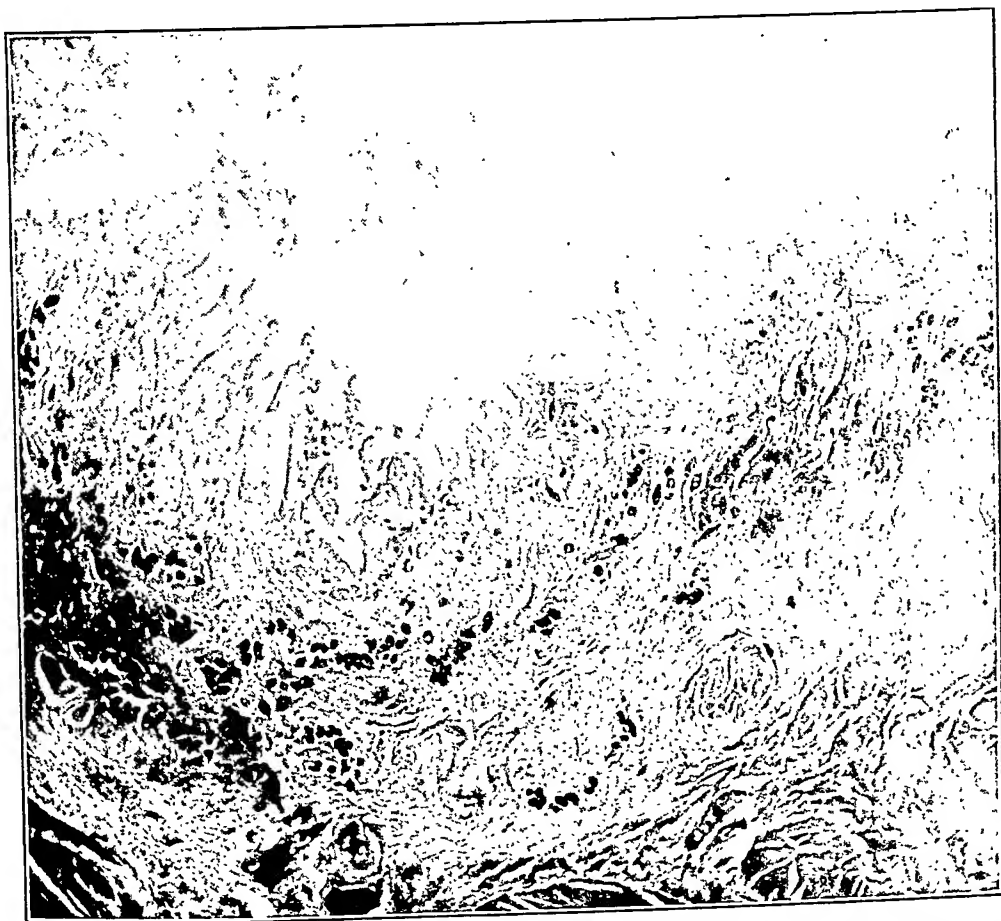
PLATE 5

FIG. 11. Pseudotubercle, late stage, with marked formation of fibrous tissue.

FIG. 12. Bladder wall showing dense infiltration with ova of *S. hematobium*, many of which are calcified.



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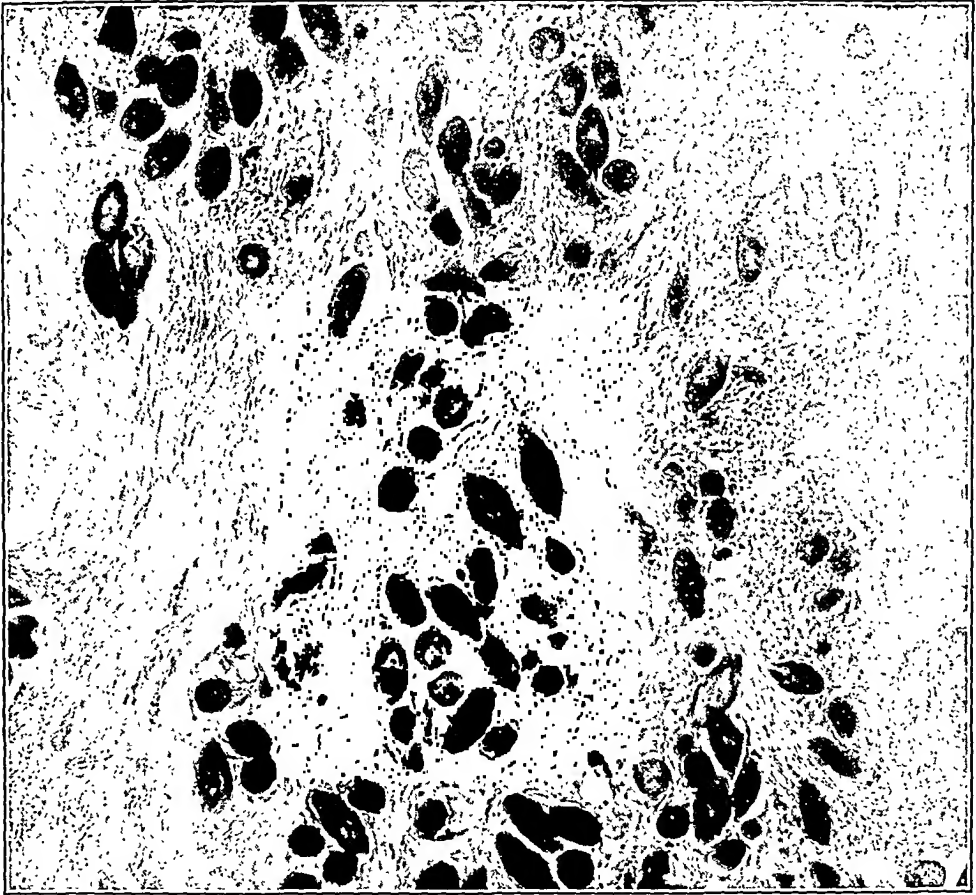


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PLATE 6

FIG. 13. Terminal spined ova of *S. hematobium* in the bladder wall, (comparatively low magnification).

FIG. 14. Mucinous degeneration in the columnar epithelium on a rectal polyp. The dark spot under the mucosa is the cross-section of an ovum.



13



14

A STUDY OF THE CIRCULATION IN THE NORMAL AND PATHOLOGIC KIDNEY WITH ROENTGENOGRAPHIC VISUALIZATION OF THE ARTERIAL TREE, INCLUDING THE GLOMERULI *

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INTRODUCTION

Our knowledge of the structure and physiology of the kidney has steadily progressed in recent years, but the methods employed by the workers in these fields have not been adopted quite so vigorously by the student of general pathologic conditions of the kidney.

Oertel¹ in 1910 maintained that in order to establish a pathologic physiology of the kidney it would be necessary to "construe the plan of the whole pathological organ." Not until the recent work of Traut,² however, has it been possible to interpret accurately the plan of the kidney from an anatomic standpoint, and this investigator is convinced that the unit structure of the kidney is largely explainable on the basis of a unit blood supply. It would seem therefore that a study of a series of kidneys from necropsy material by some method which clearly, accurately and completely visualizes the arterial tree, might offer a valuable contribution toward our conception of renal pathology and its relationship to functional capacity.

HISTORICAL

Studies of the renal vascular tree have been made by numerous workers using celloidin and corrosion methods, but with such a procedure one is handicapped from a pathologic standpoint by the unavoidable destruction of the kidney tissue.

The use of a radiopaque injection mass for the study of the circulation dates back almost as far as the use of the Roentgen rays for diagnostic purposes. The earliest investigations are credited to V. Dutto, an Italian, who in 1896 employed in his studies a suspension of calcium sulphate in water. Since that time a large number of substances have been tried by different investigators for this purpose, a concise résumé of which is given by Gough.³

* Received for publication August 15, 1927.

In the study of the circulation of the kidney by these methods the earliest comprehensive work appears in 1913 by Hauch ⁴ who used a suspension of red lead oxide in paraffin oil. By this means he obtained beautiful and accurate radiographic reproductions of the arterial tree. He mentions the fact that glomeruli were injected, but these are lost in the reproductions accompanying his article, the injection not extending beyond the interlobular arteries. Although he demonstrated moderate and severe degrees of renal arteriosclerosis, yet his study was preliminary in character as he gave particular attention to the method and no conclusions were drawn from a pathologic standpoint.

In 1917 and 1918 Gross, ⁵ using gelatin preparations of Prussian blue and carmin, made a roentgenographic study of normal and arteriosclerotic kidneys with a gelatin barium sulphate injection mass. This preparation gave a clear-cut reproduction of the arterial tree extending through but not beyond the interlobular branches. More recently Hinman, Morison and Lee-Brown ^{6,7} used a thin suspension of barium sulphate in a 50 per cent aqueous solution of sodium bromide to demonstrate this portion of the vascular tree, but found the particles in the mass too large to go beyond the interlobular arteries. For demonstration of the afferent and efferent glomerular circulation they substituted a celloidin corrosion method with excellent results. This latter method while admirably adapted to an anatomic study is less adaptable to pathologic work, not only because of the delicate, time-consuming procedure, but also in that it destroys the tissue and precludes histologic examination.

METHOD

For our purpose a radiopaque mass developed by Hill ^{8,9} in 1921, published by him in 1924 and completed in 1927, appeared most suitable. As prepared by him, the preparation for general use consists of a 17 per cent suspension of bismuth oxychloride (a special brand) in water containing 10 per cent acacia. A number of preliminary tests with this material indicated that a preparation of 25 per cent bismuth with 12 per cent acacia visualized both the large vessels and glomeruli more satisfactorily. A microscopic study of the individual particles in the suspension as prepared in this manner showed them to be quite uniform in size and shape, measuring

from 0.5 to 3 microns; however, these particles tend to become loosely held together, appearing as irregular masses varying from 6 to 60 microns. The larger of these apparent clumps might well be expected to obstruct branches of relatively large vessels causing artefacts, but neither microscopic kidney sections (Figs. 2, 3, 4) nor magnification of the roentgenograms indicated any such condition.

A more detailed report as to the preparation and technic is deemed unnecessary and undesirable because of the simultaneous publication by Hill of the entire method⁹; particularly as the percentages of bismuth oxychloride and acacia given above apply to renal studies and would occasion difficulties if applied to other circulatory research.

Pathologic Material: The study was made on a series of forty-eight kidneys obtained from the routine necropsies performed at the Pennsylvania Hospital during the winter and spring of 1927. As a rule the right kidney was chosen and after injection was preserved for further gross or histologic examination.

Technic: It seemed desirable that the procedure should be as simple as possible and without elaborate apparatus, so as to be applicable to the exigencies of the necropsy room. After a few trial injections with the organ *in situ*, this method was abandoned and the following procedure was adopted. The renal artery was cut at the aortic junction and the kidney was then removed in each case with the surrounding perirenal fat and the adjacent suprarenal gland. The organ was immediately placed in the ice-box in physiologic saline solution until an opportunity for injection was afforded. In this way kidneys were injected for the most part a few hours after death, but in some cases a longer time elapsed. In one instance a kidney was kept in this manner for about eight days and to our surprise the prolonged delay did not seem to affect the subsequent study. When ready to inject, the organ was brought to room temperature and the renal artery was ligated under saline, any air thus being excluded from the vessels. The injection mass was introduced into the renal artery with a 20 cc. syringe and hypodermic needle, using gentle digital pressure. Leaking vessels were caught with hemostats when necessary. By placing the tissue immediately in saline, the air is not only excluded from the vessels but injection into the saline-filled system dilutes the first part of the suspension, so that the same effect is produced as if a thin suspension was fol-

lowed by a heavier one. It is felt that the cold saline also probably prevents the formation of clots and possibly helps to dissolve them.

The injection was carried out carefully and slowly, requiring from five to twenty minutes. It was considered complete when there was an even distribution of tiny white points visible through the kidney capsule. In several injections just as the procedure was completed the return venous flow of blood showed a white tinge of the injection mass, but in no instance was a sufficient quantity of the mass passed through the capillaries to visualize the veins. Where infarcts or cortical scars were present these areas refused to fill out either with prolonged gentle pressure or greater pressure. The fatty renal fascia was then stripped off, any leaking points clamped and tied if necessary, and any injection material on the surface washed off carefully in cold water and the organ dried with a towel. Stereoscopic roentgenograms were taken as soon as convenient using a low milliamperage and low spark gap technic. In a few instances where the injected specimen was not roentgenographed for eight to fifteen hours after injection, the settling out in the larger vessels produced artefacts.

The amount of material injected proved to be fairly constant (where leakage was negligible) ranging from 7 to 18 cc., and averaging from 12 to 15 cc.; kidneys of children and contracted kidneys taking a smaller amount, and large kidneys, especially those with passive congestion, taking a larger quantity.

RESULTS

General: Roentgenograms of the injected kidneys gave a clear-cut reproduction of the arterial tree including the glomeruli, coinciding accurately with the present conception of its anatomy so clearly presented by Lee-Brown.⁷ In a single instance, which will be discussed later, the pyramidal vessels were injected (Fig. 10). In each instance the films were studied both stereoscopically and separately with a hand lens. Stereoscopic examination proved to be of particular value in the study of the coarser vascular tree, the pyramids appearing as well defined hollows, between which the interlobar branches could be easily traced with the arcuate arteries arching over their bases. The relationship of areas of infarction, scars, metastatic nodules, tubercles, and artefacts was also best under-

stood by stereoscopic examination. The finer vessels were studied to best advantage with a hand lens although even with the naked eye the straight, almost parallel interlobular arteries could be readily seen surrounded by compact areas consisting of many tiny points representing the glomeruli (Fig. 8).

For further description of the findings, the cases are divided into the following groups: I. *Normal arterial tree*; II. *Arteriosclerosis* (those two groups have been based primarily on the roentgenographic appearance); III. *Thrombosis, infarction and metastatic tumors*; IV. *Syphilis*; V. *Tuberculosis*; and VI. *Hydro- or pyonephrosis*. Group I includes eighteen cases in which no pathologic change was demonstrable in the arterial tree. Group II is composed of seventeen cases in which there was some degree of generalized arteriosclerosis. Group III consists of six cases showing thrombi or infarcts in the kidney and one case in which there were multiple metastatic tumor nodules. Group IV includes six cases of syphilis, which are considered separately because of the often discussed question as to whether changes in the small vessels in such cases are of syphilitic origin or merely represent simple arteriosclerosis. Group V includes four cases of generalized miliary tuberculosis. Group VI is composed of four cases of hydro- or pyonephrosis which were encountered in the series.

GROUP I. *Normal Arterial Tree*. In the normal arterial tree the primary division of the renal artery appears on the film either just within or lying closely outside the edge of the cortex at the hilum. The vessels show a uniform gradual diminution in the size of their lumina as they progress from the hilum toward the cortex. Except for the primary branches arising from the renal artery the branches come off at an acute angle. The interlobar and arcuate branches, passing up between the pyramids, spread out in graceful curves and arches over the bases of the pyramids not unlike the architecture of a great elm tree (Figs. 5, 6, 7 and 8). The interlobular arteries are slender, delicate, of uniform size, very straight, almost parallel and give rise to a picture of a uniformly thick vascular cortex. The glomeruli are very fine, numerous and uniform in size. They are grouped closely around the interlobular arteries giving the effect stereoscopically of slightly cone-shaped columns with the interlobular artery in the center and their bases toward the periphery of the cortex (Fig. 13).

It is of interest to note the variety of pathologic conditions in which injection showed a normal arterial tree. To establish our conception of the normal, particular attention was given to cases in which the age of the individual was within the first three decades of life. Of two in the third decade, one, age 24, died of generalized melanomatosis (Fig. 13); the other age 27, died of vegetative endocarditis (Fig. 8) and will be referred to again in a later group. Two cases of rheumatic fever are representative of the second decade, one in the acute (Fig. 5) and one in the chronic stage. The patients in both of these cases were 14 years of age. A single case of a patient 8 years of age, who died of extensive burns of the body, represents the first decade (Fig. 7). Cases showing infarcts, thrombi, or metastatic tumor nodules are included in Groups I or II depending on the appearance of the arterial tree on the films.

With kidneys from individuals in mid-life the incidence of demonstrable vascular disease is so high that selection of cases is more difficult. Five cases were listed as normal in which the ages vary from 40 to 51 years, and in which there were no appreciable arterial or arteriolar changes microscopically. On the roentgenograms the arterial tree appears somewhat less delicate than in the series of younger individuals. The glomeruli appear to be of approximately the same size and number, and the cortex of the same width, but the arcuate and interlobar branches show more abrupt changes in size of the lumina while the curves and arches lose some of their gracile, slender form (Fig. 6).

Among the conditions in which a normal vascular tree was visualized were three cases of malignant tumor, a variety of infectious conditions and one case of subacute nephritis (Fig. 9). In this series we are confronted with various degrees of passive congestion and cloudy swelling in the kidney, with one case presenting an active nephritis. To our surprise none of these factors appeared to influence or to limit the completeness of the injection. Figure 5 shows a kidney with marked passive congestion from a case of cardiac failure in active rheumatic fever. The injection is heavy, uniform, and the injection mass is beginning to enter the pyramidal vessels. Figures 9 and 14 show a roentgenogram of a kidney which histologically showed not only swelling of the tubular epithelium but also an active nephritis. Grossly this kidney was a typical example of subacute nephritis or so-called "large white kidney," yet the injection

visualizes a beautifully delicate vascular tree and from the roentgenogram it would be impossible to say that a lesion existed in this kidney.

Figure 10 is the roentgenogram of the one case in the entire series in which an injection of the pyramidal vessels is obtained which approximates completeness although some degree of pyramidal injection was obtained in several instances. The reason for this is somewhat obscure since the same technic was used. The case was one of gangrenous endometritis following an abortion, in a woman 33 years of age, who presented a profound anemia together with evidences of severe toxemia. At necropsy there was a severe toxic hepatitis and the kidneys showed marked cloudy swelling and passive congestion. The blood urea nitrogen (postmortem) was not, however, increased. The possibility presents itself that the vessels were unusually deficient in tone due to the toxic phenomena and therefore permitted a more complete penetration of the injection mass into the finer vascular tree. The pyramidal arterioles are very straight, but wide and feathery converging toward the tip of the pyramid. From the base of each pyramid delicate vessels can be distinguished running up into the cortico-medullary zone which are very tiny but not as straight as the interlobular arteries among which they lie (Fig. 15).

GROUP II. *Arteriosclerosis*. The changes in the coarser portion of the vascular tree have been visualized by Hauch and later by Gross, the latter tabulating them in detail. In the advanced stages of arteriosclerosis of the kidney associated with a generalized contraction of the entire organ the changes are most striking. The lumina, especially those of the larger vessels, are of very uneven caliber presenting many constrictions and irregularities due either to plaques in the intima or thickening of the walls, while adjacent areas show either real or apparent dilatation. The branches tend to arise more nearly at a right angle. The interlobar and arcuate arteries are wider, and instead of tapering gradually toward the cortex maintain a rather large caliber and end abruptly in smaller branches. The normal curves have become angular, and the vessels tortuous in their course. The interlobular arteries are fewer, shorter, and run less directly to the cortex, and in severe cases even exhibit a tortuous course (Fig. 11). The glomeruli are fewer in number, considerably enlarged and are seen clumped around areas of the richest

surviving vascular supply (Figs. 11 and 16). The entire picture of the arterial tree is that of a gnarled, dead oak, rather than the graceful architecture of the spreading elm tree. As the kidney contracts it shrinks uniformly but the coarser arterial tree maintains the same size and position, so that there is the appearance of a drawing out of the renal artery and its larger branches from the hilum.

In the milder degrees of nephrosclerosis the earliest change seen in the roentgenograms appears in the arcuate and peripheral portions of the interlobar arteries. These appear coarser and run a tortuous course (Fig. 12). In this stage the glomeruli may show no demonstrable change (Fig. 17) although in several instances small cortical defects appeared in the roentgenograms. On microscopic examination these were found to consist of scarred areas lying beneath the capsule. Histologically the vessels showed arteriolar sclerosis.

Between this and the contracted kidney first described there are varying degrees of arterial change. In all, however, the degree of change in the interlobar and arcuate arteries is a striking feature. Changes in the size and number of functioning glomeruli bear a close relationship to the condition of these vessels as seen in the picture of the arterial tree.

This series includes kidneys from sixteen cases ranging in age from 33 to 77 years. Only one case, however, was under 40 years, and half were over 50 years of age. In all except two cases, generalized arteriosclerosis was an important factor in the anatomic diagnosis and these two cases showed only mild arteriolar and arterial change in the kidneys.

Differentiation between nephritis of arteriosclerotic origin and other types of nephritis. In connection with the studies on kidneys showing advanced degrees of arteriosclerosis, it has seemed particularly interesting to determine whether this method of visualization of the vascular tree would prove of value in the differentiation between the contracted kidneys of arteriosclerotic origin (nephrosclerosis) and the contracted kidneys of essentially toxic or bacterial origin (chronic glomerulonephritis). A single specimen representing the latter type of lesion was available for this comparative study. No attempt has been made to define the picture of chronic glomerulonephritis on the basis of this isolated case but the findings are sufficiently striking to warrant their presentation. The case was that of a male negro,

44 years of age, who gave a history of failing renal function for a period of at least nine years. At necropsy the kidneys presented the picture of chronic glomerulonephritis associated with a relatively mild degree of vascular sclerosis. The roentgenogram (Fig. 18) is in contrast to that of the arteriosclerotic kidney (*cf.* Figs. 11, 16).

It will be noted that sclerotic changes in the arterial tree are of moderate degree and occur largely in the arcuate arteries. The cortex, although twice as wide as in the case of nephrosclerosis, shows only a very few, large and fairly evenly distributed, glomeruli. Interlobular vessels where distinguishable are thick and tortuous, and an almost complete injection of the pyramidal vessels is evident. This last feature appears especially remarkable in view of the apparent distortion of the normal circulation.

GROUP III. *Thrombosis, Infarction and Metastases.* This group includes seven cases; one with metastatic tumor nodules, two with thrombi, and four with infarcts. On the roentgenograms of kidneys with the arterial tree injected, infarcts and thrombosed vessels cause a ragged, irregular defect, extending down from the cortex corresponding quite accurately in size to that of the gross lesion. Metastatic tumor nodules are frequently well rounded, sometimes raised above the surface, and appear also as defects in the injection of the arterial tree. It must be remembered that scars, tubercles, and artefacts may give the appearance of thrombosis or infarction, whereas retention cysts and abscesses simulate the picture of metastatic nodules, so that a careful check by gross and microscopic study of the kidney is essential. However, the injection method appears to be of very definite value for the detection of certain lesions of this type and in our hands has, in two instances, enabled us to demonstrate recent infarction where it was impossible on gross examination of the organ to distinguish any change in the tissue in the thrombosed area and hence there was no indication for histologic study of the particular area except the defect in the roentgenograms. In three cases white infarcts were readily seen on gross examination, and the appearance of these on the roentgenograms was essentially the same as those in the cases showing earlier lesions except that the areas of infarction were somewhat less extensive (Figs. 7 and 8).

One case in which there was a small aneurysm of the abdominal aorta at the level of the renal arteries is of special interest. The right kidney was very large while the left was about one third the

size of the right and had a peculiar bulb-like enlargement of the lower pole which was supplied by a separate anomalous artery arising from the aorta. The left main renal artery was occluded by a laminated thrombus for a distance of two and a half cm. from the aorta, distal to which it was collapsed and flabby. Both kidneys were injected for roentgenography (Fig. 19). The right showed slight sclerosis of the interlobar and arcuate arteries. In the left, the lower pole was similar in appearance to the right kidney, but the upper two thirds of shrunken kidney showed small vessels, the branches tending to be drawn out at the hilum and more parallel than normal. The arcuate vessels were tortuous, almost spiral, yet without the marked clubbing of severe arteriosclerosis. The cortex was but 1 to 2 mm. in width and the glomeruli were not injected. During the injection it was noticed that a small amount of the suspension came through into the renal pelvis, which would suggest tiny breaks in the severely damaged tissue and this is further borne out by the fact that the patient had had hematuria before death. The perforating capsular arteries were remarkably large and the possibility of a very deficient anastomotic blood supply by this route is to be considered (Belt and Joelson¹⁵). The picture is evidently the result of a relatively slow occlusion of the blood supply rather than a sudden blocking of the renal artery, and stands in sharp contrast to the cases of sudden blocking of a vessel with subsequent death of the tissue in the area supplied by it.

GROUP IV. *Vascular Syphilis*. This series includes six cases, all of which exhibited syphilitic aortitis and four of which presented aortic aneurysms. Two cases, which showed aneurysms of the aorta, displayed a normal arterial tree both on roentgenograms of the injected specimens and upon gross and histologic examination. The ages of these cases were 31 and 50 years. Three cases exhibited sclerotic changes in the interlobar and arcuate arteries and their branches. The ages here ranged from 52 to 64 years. In the remaining case cerebrospinal syphilis was a prominent feature. The kidney of this patient showed marked hydronephrosis and pyelonephritis. The changes in the roentgenograms here, consisting chiefly of an elongation of the primary branches of the renal artery and the interlobar arteries with slight enlargement of the arcuate branches, were essentially those produced by hydronephrosis which have been described by Lim¹⁰ and again by Hinman and Morison.¹¹

It is impossible to draw conclusions from so small a series as to whether the high incidence of arteriosclerotic change in the renal vessels in this group is due to the common association of syphilis with simple arteriosclerosis of the medium-sized and small arteries as emphasized by Warthin,¹² or whether the fact may be accounted for by the advanced age of these patients. It is worthy of note, however, that the roentgenographic appearance of the changes in the arterial tree is similar in all respects to those noted in the series of arteriosclerotic cases. Histologically the picture is also essentially that of arteriosclerosis although in one case moderate obliterative endarteritis was present.

GROUP V. *Tuberculosis*. Out of the four cases of generalized miliary tuberculosis in the series, tuberculous lesions were found in the kidneys in only three instances. The striking feature here was the resemblance between the defects produced by tuberculous lesions and those produced by thrombi and infarcts. We were unable to distinguish the presence of tubercles in roentgenograms of injected kidneys until they reached a size of about 1 to 2 mm. in diameter and the picture then was that of tiny or small defects, apparently the result of encroachment of the spreading tubercle on surrounding capillaries, glomeruli, and arterioles. The appearance was characteristic only in respect to the widespread distribution of tiny defects, all of which were of approximately the same size. Later, when the tubercles became confluent, they appeared more as small infarcts or scars, although possibly more superficial and less likely to involve small arteries.

GROUP VI. *Hydronephrosis*. There were also four cases of hydro- or pyonephrosis encountered in the series, only one of which was of marked degree. Subsequent to injection of the arterial tree, a pyelogram was made in each of these cases, but the changes noted were constantly proximal to the interlobular arteries and the findings add nothing to the work already done on this subject (Lim,¹⁰ and Hinman and Morison¹¹).

RELATIONSHIP OF DEMONSTRABLE VASCULAR CHANGES TO KIDNEY FUNCTION

An estimate of the functional impairment of the kidneys in this series is available in thirty-one cases in which terminal urea nitrogen and creatinine determinations were made on the blood. Except in

three instances these determinations were done postmortem on blood obtained at necropsy, as it has been shown that such data are reasonably accurate within a small percentage of error when the blood specimen has been taken within twenty-four hours after death (Paul ¹³).

The cases in which blood urea nitrogen determinations are available are fairly evenly distributed throughout the groups (Table I).

TABLE I

Showing the Percentage of Cases with High and Low Blood Nitrogen Figures in each Group

Group	Total cases	Number of cases with urea nitrogen and creatinine determination on blood	Cases with normal blood urea nitrogen		Cases with high blood urea nitrogen	
			Number	Per cent	Number	Per cent
I. Normal	18	9	3	33	6	66
II. Arteriosclerosis. . .	17	14	4	28	10	71
III. Infarcts	7	4	0	0	4	100
IV. Syphilis	8	5	1	20	4	80
V. Tuberculosis	4	4	2	50	2	50

In the two larger groups (I and II) it is surprising to find that the percentage of cases showing a high blood urea nitrogen is almost as great in the group of so-called normals as in the arteriosclerotic series. Moreover, if we exclude from the later group one case of severely contracted kidney in which there was an active nephritis at death, and from Group I the case of subacute nephritis, it is found that the limits of the blood urea nitrogen are only slightly higher in the arteriosclerotic group. Furthermore, there does not appear to be any appreciable difference in terminal kidney function between the cases of fairly marked arteriosclerosis and those of moderate degree. This might appear to be unusual, yet it is recognized that in kidneys with moderate diffuse or focal scarring there is compensatory hypertrophy of secretory tissue and consequently the functional capacity may be relatively unchanged; and, as MacCallum ¹⁴ has suggested, "Perhaps the majority of the cases in which the pathologist at autopsy writes down 'slight chronic nephritis' are only instances of such obsolete scars in the kidney whose cause is now impossible to tell." Consequently in the present series of cases it is apparent that the functional impairment present in the moderately arteriosclerotic group and also in the normal group, is, in all probability, largely due

to toxic influences associated with systemic disease rather than to demonstrable vascular changes. It has already been pointed out that most of the acute parenchymatous changes in the kidney do not appreciably affect either the injection method or the appearance of the arterial tree.

In the infarction group it is noteworthy that the blood showed some degree of urea nitrogen retention in every case. Although this suggests that infarction is responsible for lowering the functional efficiency, the series is too small to warrant such a conclusion.

In the syphilis group the percentage of normal blood urea nitrogen cases is somewhat lower than in Groups I and II, but, with the exception of one case associated with pyonephrosis, the blood urea nitrogen does not reach as high a figure as in either of the former groups.

A study of the urinalysis records on the entire series contributed nothing of further significance.

SUMMARY

1. The injection of the renal vessels with a radiopaque suspension, and subsequent roentgenography of the kidney have proved a simple, easily accomplished procedure readily adaptable to the necropsy room as a means of reconstruction of the arterial tree including the glomeruli. This method does not interfere with the usual methods of gross and microscopic examination.

2. Roentgenographic results check accurately with the accepted anatomic conception of the arterial tree; and in the normal kidney the vessels show:

- (a) A slender, gradually decreasing caliber of the lumen.
- (b) Branching at an acute angle except at the primary division of the renal artery.
- (c) Interlobar and arcuate arteries with gradual curves and arches like a spreading elm tree.
- (d) Interlobular arteries which are slender, delicate, closely set and almost parallel, giving a thick uniform cortex.
- (e) Glomeruli that are very fine and numerous, grouped evenly around the interlobular arteries, giving an appearance stereoscopically of slightly cone-shaped columns with the interlobular artery in the center and their bases toward the periphery of the cortex.

3. Severe arteriosclerosis is marked by:
 - (a) An uneven caliber of vessels with dilatations and constrictions.
 - (b) A branching angle which approaches a right angle.
 - (c) Interlobar and arcuate arteries which are wider, club-like and do not taper gradually. Their curves are abrupt and angular.
 - (d) Interlobular arteries which are fewer, shorter, tortuous, and do not run so perpendicularly to the cortex.
 - (e) Glomeruli that are larger, decreased in number, and clumped around areas of best vascular supply.
 - (f) With contraction of the kidney the cortex thins and the entire vascular tree appears drawn out from the hilum, and the picture of the arterial tree as a whole suggests a gnarled oak rather than a graceful elm.
 - (g) Milder degrees of sclerosis are found first in the periphery, *i. e.*, in the arcuate and interlobar arteries, and extend farther toward the hilum as the changes become more marked.
4. The vascular changes in syphilis, when present, were of the same appearance as those of arteriosclerosis in this series.
5. Examination by the injection method is of definite value in the study of thrombosis of vessels and infarcts.
6. A comparison of the roentgenographic appearance of the arterial tree with the urea nitrogen retention in the blood has failed to show a direct relationship between moderate arteriosclerosis *per se* and impaired renal function.
7. Acute nephritis, acute degenerative parenchymatous changes in the kidney tissue, and passive congestion have neither interfered with the injection procedure nor have they given a distinctive picture on the roentgenograms.

I wish to thank Dr. Eben C. Hill of the Department of Anatomy of Johns Hopkins Medical School for his kind assistance in the adaptation of his injection mass to this special work, without which the study would not have been possible.

I am also greatly indebted to Dr. J. R. Paul, Director of the Ayer Clinical Laboratory for his encouragement and constructive criticism throughout this work and to Drs. D. R. Bowen and P. A. Bishop of the Department of Roentgenology of the Pennsylvania Hospital for their kindness and coöperation in doing the roentgenography.

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DESCRIPTION OF PLATES

PLATE 7

- FIG. 1. Photograph of section of kidney showing appearance after injection. The specimen was cut, washed, and fixed in Klotz' solution so that the injected mass has been washed out of the larger vessels; but tiny vessels in the cortex and pyramids are well seen as fine lines, and the glomeruli appear as punctate points. See Fig. 6 for roentgenogram.
- FIG. 2. Photomicrograph (very low power) of injected renal cortex showing the injection mass in interlobular arteries, in glomerular arteries and in glomeruli as dense black material. Note the anatomic arrangement of glomeruli around the interlobular artery.
- FIG. 3. Photomicrograph (low power). Portion of an interlobular artery showing glomerular arteries arising from it and glomeruli filled with the injection mass.



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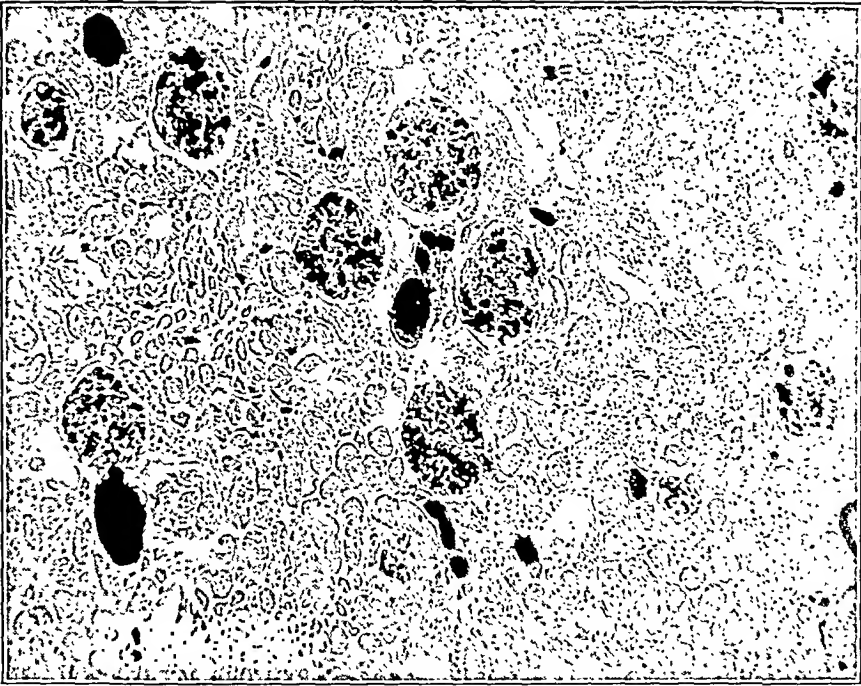
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Circulation in Normal and Pathologic Kidney

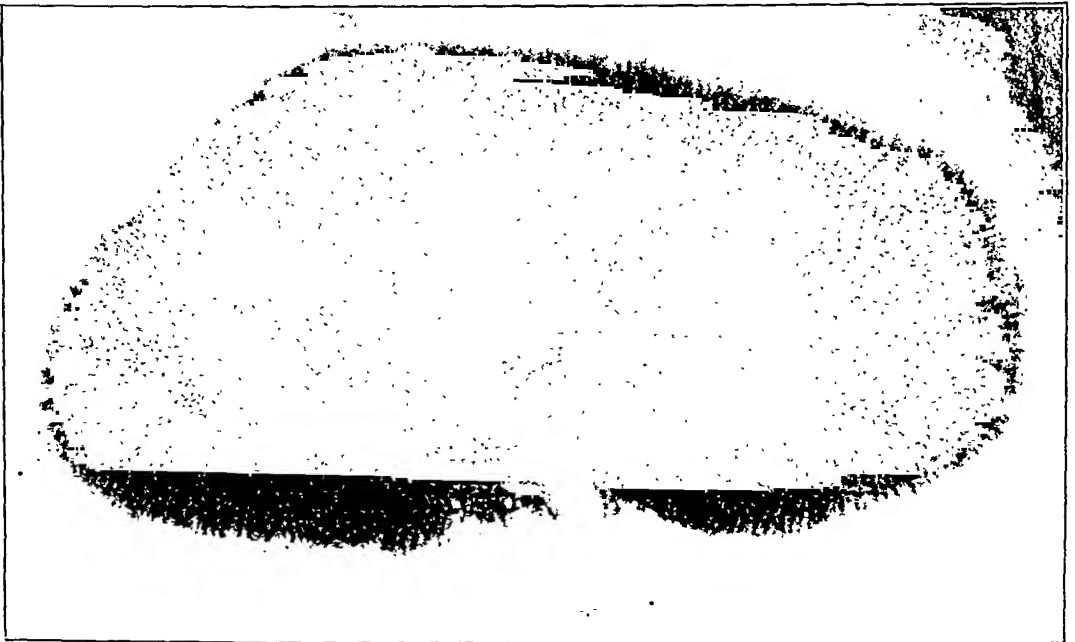
PLATE 8

FIG. 4. Photomicrograph (low power). A tangential section of the cortex showing the injection mass in interlobular arteries and glomeruli. Note the grouping of glomeruli around the interlobular vessels. The histologic study is not interfered with by the injected material.

FIG. 5. Roentgenogram of a normal arterial tree. Kidney from a boy, 14 years of age, dying of cardiac decompensation in active rheumatic fever.



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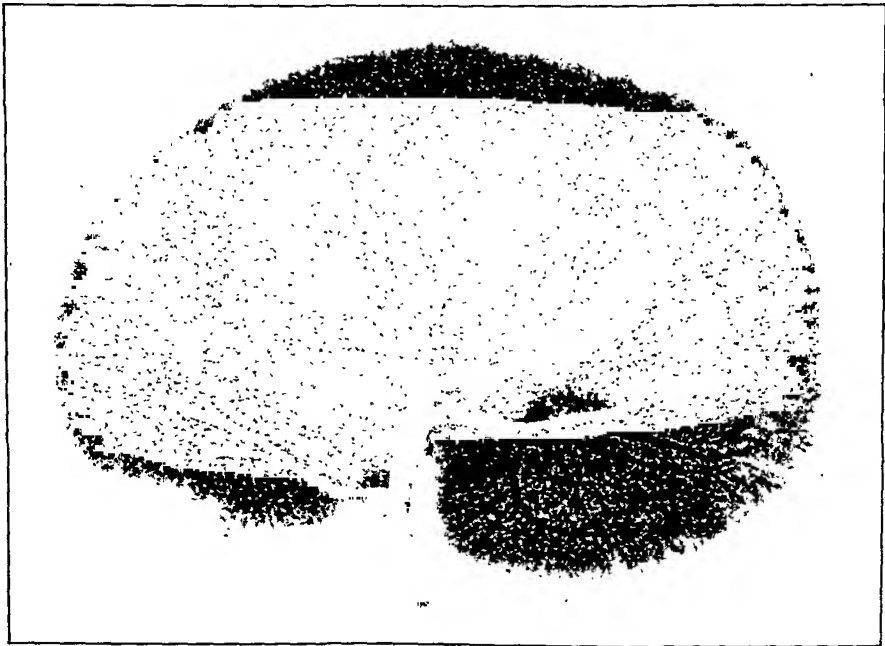
PLATE 9

FIG. 6. Roentgenogram of a normal arterial tree. Kidney from a man, 40 years of age, dying of carcinoma of the liver. The apparent constriction of the origin of one of the large primary divisions of the renal artery is found stereoscopically to be due to an optical phenomenon on the flat view.

FIG. 7. Roentgenogram of a normal arterial tree with infarcts. One infarct is seen at the periphery of the cortex near the hilum as an irregular defect and another as a dark area in the middle of the opposite pole. The hollows of the pyramids are well seen. Kidney from an 8-year-old girl dying twenty-eight days following extensive third degree burns of the body and extremities.



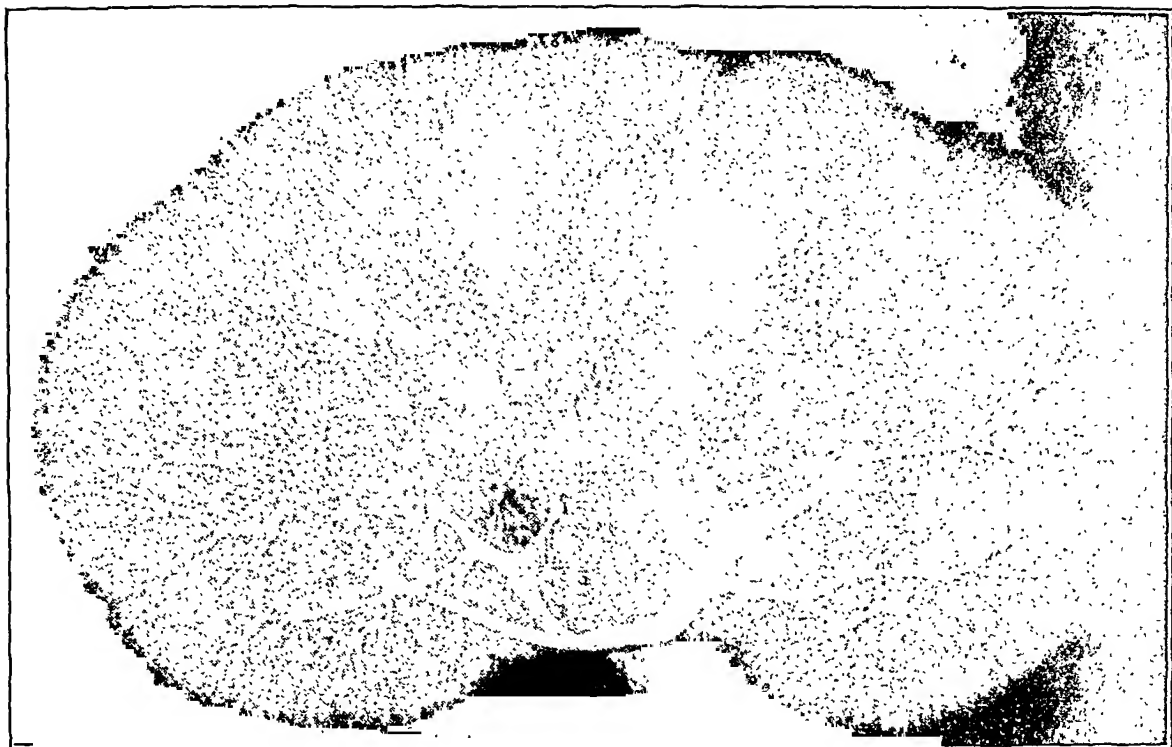
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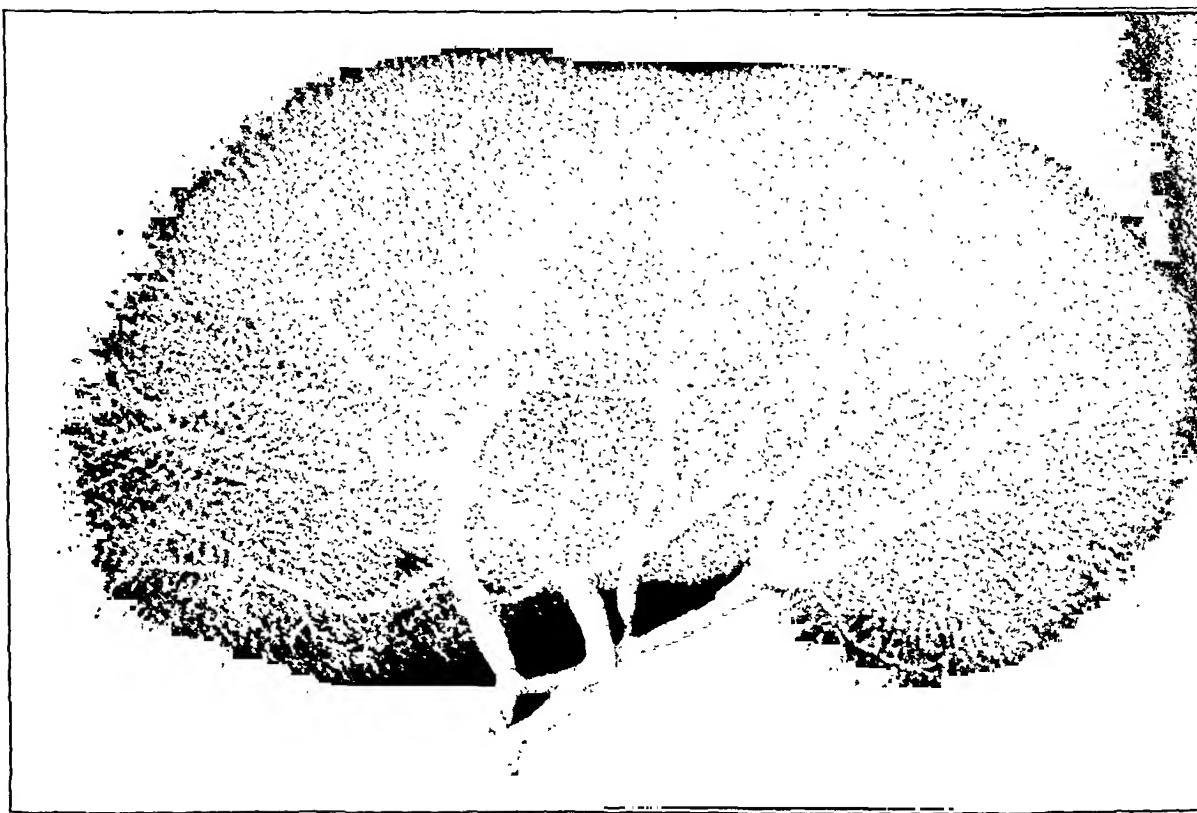
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PLATE 10

- FIG. 8. Roentgenogram of a normal arterial tree with infarcts. Three rather large defects in the cortical injection are seen at the periphery of the upper pole, and two deep in the region of the lower calyx near the hilum. These were white infarcts grossly. Kidney from a patient 27 years of age dying of vegetative endocarditis.
- FIG. 9. Roentgenogram of a kidney with subacute nephritis, "large white kidney." The arterial tree is normal and the injection complete and heavy.



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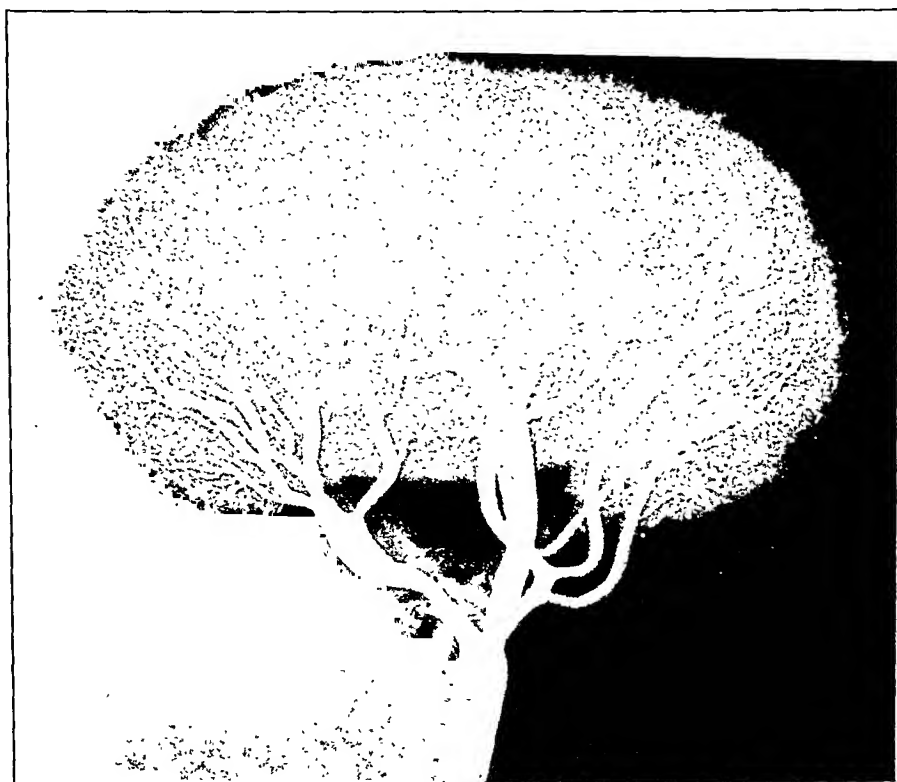
PLATE II

FIG. 10. Roentgenogram of a kidney showing apparently complete injection of the pyramidal vessels. The arterial tree is normal. Kidney from a woman, 33 years of age, dying of gangrenous endometritis. Histologically there was marked passive congestion and cloudy swelling.

FIG. 11. Roentgenogram of a severely contracted kidney (nephrosclerosis). Note the uneven lumina and "dead tree" appearance of the vessels and the relatively few and very large glomeruli. A large retention cyst is seen on the periphery opposite the lower border of the hilum. Kidney from a man, 55 years of age, having generalized arteriosclerosis and dying of uremia.



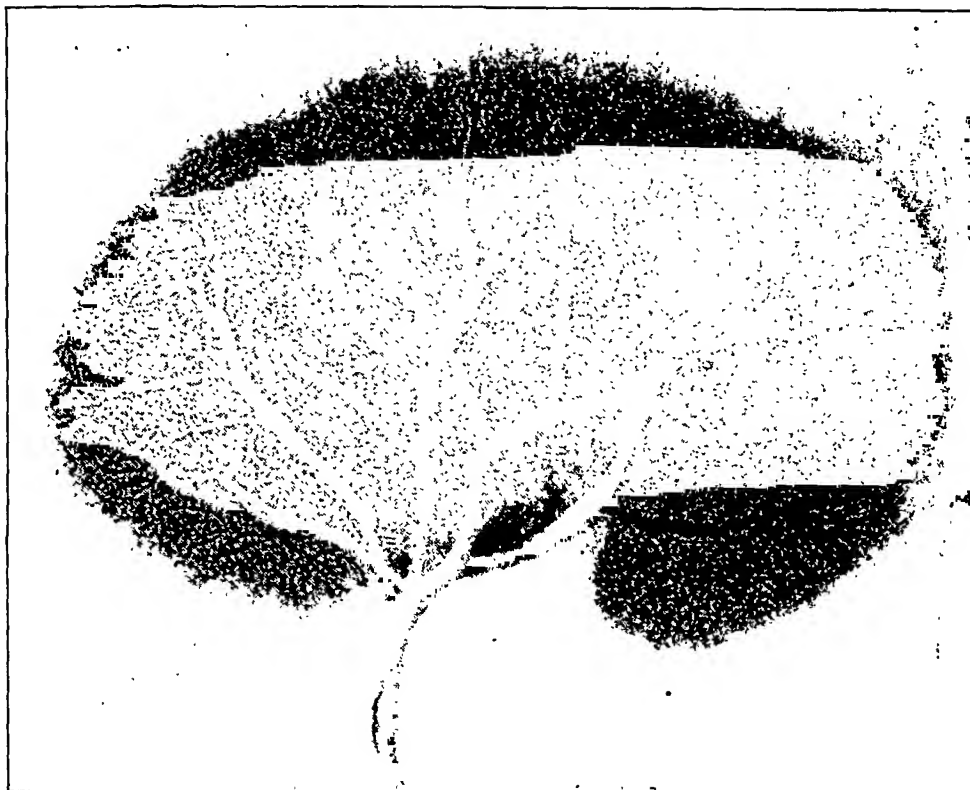
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PLATE 12

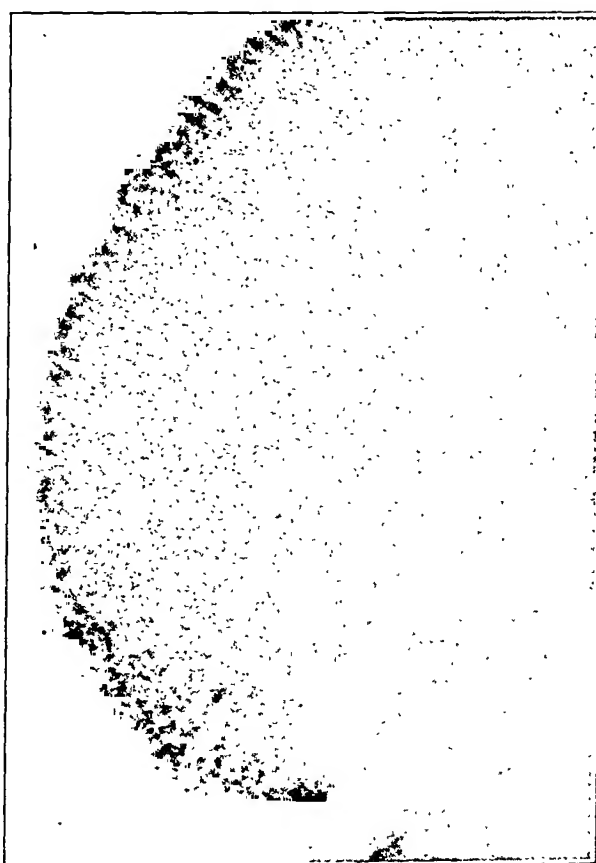
- FIG. 12. Roentgenogram of a kidney, the vessels of which show moderate arteriosclerosis. Changes are most marked peripherally, the cortex is narrow, and the arcuate and interlobar vessels are tortuous with widened lumina, and end abruptly. Kidney from a woman, 70 years of age, with generalized arteriosclerosis, dying of cerebral thrombosis with infarction.
- FIG. 13. Roentgenogram (small area enlarged $2\frac{1}{4}$ times) of normal arterial tree to show the details of the delicate tapering structure of the interlobar and arcuate arteries, and the small, very straight interlobular arteries almost hidden by surrounding clumps of glomeruli.
- FIG. 14. Small area of roentgenogram of Fig. 9 (enlarged $2\frac{1}{4}$ times). The thick cortex shows the column-like appearance produced by glomeruli clumped around the interlobular arteries.



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PLATE 13

FIG. 15. Small area of roentgenogram of Fig. 10 (enlarged $2\frac{1}{4}$ times). The vessels of the pyramids, broad and feathery at the base and narrowing as they converge toward the tip, are well shown.

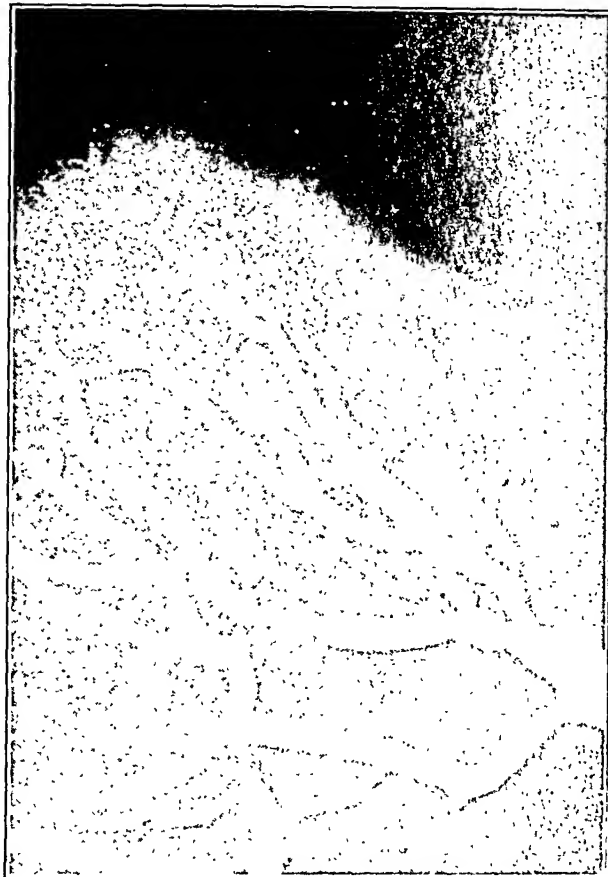
FIG. 16. Small area of roentgenogram of Fig. 11 (enlarged $2\frac{1}{4}$ times). Note the few and very large glomeruli in the narrowed cortex.

FIG. 17. Small area of roentgenogram of Fig. 12 (enlarged $2\frac{1}{4}$ times). The tortuosity and uneven dilatation of the lumina of interlobar and arcuate arteries is clearly seen. The glomeruli do not appear increased in size.

FIG. 18. Roentgenogram (small-area enlarged $2\frac{1}{4}$ times) of a contracted kidney of chronic nephritis. Note the relatively mild sclerotic changes in the arterial tree, the rather wide cortex containing but few greatly enlarged glomeruli and the fairly complete injection of the pyramidal vessels. Compare the appearance here with that of a contracted nephrosclerotic kidney in Fig. 16.



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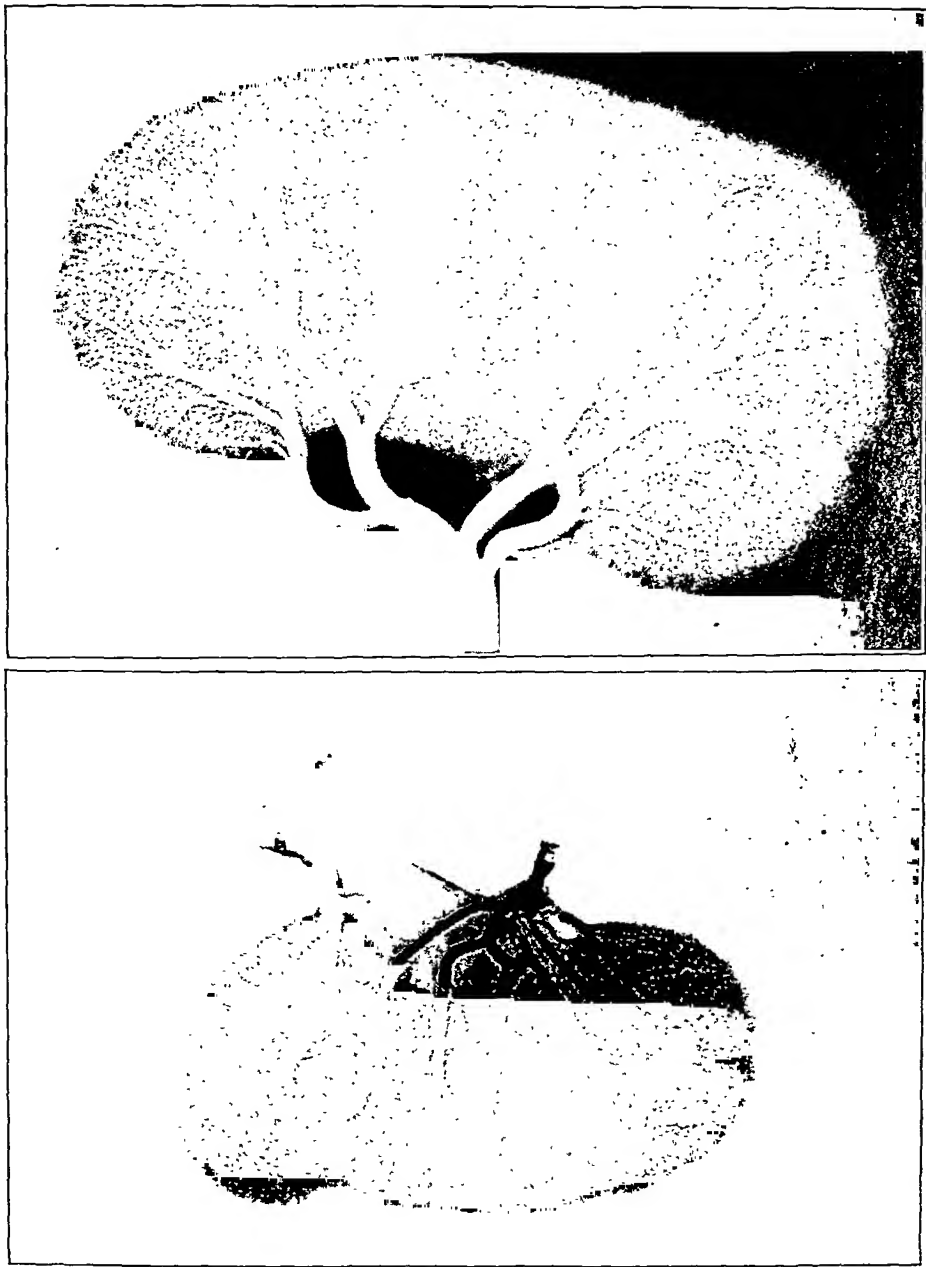
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Circulation in Normal and Pathologic Kidney

PLATE 14

FIG. 19. Roentgenograms of both kidneys from a case of aneurysm of the aorta at the level of the renal arteries. The left renal artery was thrombosed and injection distal to the thrombus in the upper two thirds, shows a shrunken kidney and a shriveled arterial tree with no glomeruli injected. The lower pole supplied by an anomalous artery is larger and normal in appearance and the line of division is sharply drawn. The renal pelvis is faintly visualized by some of the injection mass which appeared in the pelvis during the arterial injection.

The right kidney is hypertrophied. Well defined hollows are seen where pyramids (uninjected) occur. There are mild sclerotic changes in the arcuate and interlobar arteries. The patient was 45 years of age.



THE ETIOLOGY AND PATHOLOGY OF PYELITIS CYSTICA, URETERITIS CYSTICA AND CYSTITIS CYSTICA *

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Cysts of the mucous membrane of the renal pelvis, ureters and urinary bladder have long been recognized. They rarely produce clinically recognizable phenomena but among pathologists and surgeons their etiology, occurrence and evolution have been much in dispute. This study was undertaken to review and correlate these different opinions.

REVIEW OF THE LITERATURE

In the pathologic work of Morgagni²⁸ in 1761 is found the first reference to this condition. He reported two cases, one in a man aged 60 and the other in "a decrepit old man who for many years had been afflicted with lues venerea." In both cases there was bilateral hydronephrosis and hydro-ureter. He described his observations in the first case: "on feeling the ureters, the sensation of there being calculi in some parts of them was communicated, but when they were slit open, an hydatid was found in every one of the places. Some were round, others were oval, hanging from the inner coat into the canal of the ureter, but not by a small stalk. The round ones were equal in size to very small grapes and the oval hydatids were double the length of the others."

The next case to be found in the literature was reported by Johnson²⁰ in 1816. His case was that of a woman; hydronephrosis and hydro-ureter were present on the same side as the cysts.

Rayer,³⁴ in 1837, and Rokitsansky,³⁵ in 1861, reported cases of this condition. Rokitsansky says: "Cysts appear to be more frequent in the urinary passages than they are in and upon other excretory ducts. Without referring to the other cases, we may notice two that have

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been observed in the Vienna Hospital. They represent cysts, the size of millet seeds or peas, developed under the mucous membrane and either grouped together or solitary, containing a colorless or yellowish serous fluid in which is found a soft glutinous or hard nodule varying in size and resembling amber. These cysts and their mucous covering occasionally burst, which is proved by the concretions having been discovered unattached in the bladder. They were chiefly found occupying the ureters and in one case the pelvis and calices of the kidney." Neither Rayer nor Rokitansky expresses definite views as to the origin of the cysts.

Virchow,⁴³ in 1863, described a case and compared the cysts to the mucous cysts of the vagina.

Egli,¹¹ in 1873, in a study of the histology of the renal pelvis, described flat globular glands resembling sebaceous glands, but these were inconstant in their occurrence.

In 1876 Litten²⁴ offered the first microscopic description of these cysts, but failed to see or make note of any cell nests. He was definite in his conclusions that these cysts were formed by an inflammatory blocking of the mucous glands and crypts of the mucous membrane, the retention of the secretion in these glands and crypts forming the cysts.

Hamburger,¹⁵ in 1880, also described glands of the renal pelvis and ureters but stated that they were not a constant structure.

Ebstein,¹⁰ in 1882, reported a case of cysts of the renal pelvis and ureter and supported Litten's views concerning their origin.

Limbeck,²³ in 1887, reported a case of cysts of the ureter. He was unable to find glands in the mucous membrane of the ureter and concluded that the cystic formations in his case were due to the agglutination of the proliferated mucous membrane and also to the central degeneration and liquefaction of sprouts of epithelium from the deeper layers of the mucous membrane. He did not deny that cysts are formed by the inflammatory blocking of mucous glands.

In 1889 Eve¹² published the first article in English literature on this condition. He reported a case and described ovoid bodies constantly found in the colloid contents of the cysts, which corresponded in size and appearance to pseudonavicellae. This observation convinced him of the parasitic origin of these cysts. He was supported in this view by Bland-Sutton,⁵ Clark,⁷ Voelcker,⁴² Pisenti,³² von Kahlden²¹ and Morris.²⁹ In Osler's³¹ textbook, as late as the ninth

edition in 1920, psorospermiasis of the kidneys and ureter is mentioned.

Silcock³⁸ was the only English writer up to 1889 who disagreed with the theory of parasitic origin. He described foci of epidermal cells in which a lumen was formed by degeneration of the central cells.

In the meantime, von Brunn⁶ published his noteworthy article. He described epithelial bodies lying below the mucous membrane. Those showing an epithelial connection with the surface epithelium he designated epithelial buds or sprouts and those showing no connection he designated epithelial cell nests. He stated that these epithelial nests and buds were formed by the downward proliferation of the epithelium in the form of sprouts, and their subsequent separation from the mucosal lining. He believed that this proliferation of the epithelium was stimulated by inflammation. In proof of these statements, he said he was unable to find glandular elements, that the epithelium forming the nests was similar in every respect to the surface epithelium, and there was no constant lumen or exit duct, nor were the changes ordinarily seen in secretory epithelium present in the cells. The cystic formation he believed to be due to the degeneration of the central cells of the nests.

Lubarsch²⁶ shortly afterward confirmed the observations of von Brunn and reported two cases of ureteritis cystica and ten cases of cystitis cystica. He said that the microscopic appearance and the staining reaction of the contents proved that cysts were formed by degeneration of the central cells. In his cases, he found cysts originating in three separate ways: (1) from von Brunn's cell nests; (2) from the inflammatory closure of the crypts of the mucous membrane, and (3) apparently from abnormally placed glands of the upper urethra.

Aschoff³ reported a case of ureteritis cystica and agreed with Lubarsch in his views regarding the formation of cysts from von Brunn's cell nests. He examined the ureters and bladder in a boy aged $1\frac{1}{2}$ years, and a girl aged 3 years, but was unable to find either epithelial buds or cell nests or cysts. On further study, he was unable to find glandular formations above the internal sphincter of the urethra. He concluded that von Brunn's cell nests were of inflammatory origin and showed a predilection for the calices of the renal pelvis, the ureteropelvic juncture and the neck of the bladder. He

believed that these nests of von Brunn were formed in two ways: by proliferation downward of the deeper layers of the epithelium of the urinary tract, and by proliferation of the fibrous connective tissue which grew upward and cut off islands or nests of the epithelium.

Marckwald²⁷ found ureteritis and cystitis cystica in a new-born child and maintained that an inflammatory process was not necessary to the formation of von Brunn's epithelial buds or cell nests.

Stoerk,³⁹ after a thorough study of thirteen cases of ureteritis and cystitis cystica, differed with Marckwald in that he considered the origin of von Brunn's cell nests to be inflammatory. He also disagreed with von Brunn, Lubarsch and Aschoff and considered the cystic formation to be a secretory phenomenon.

Following this, Radtke,³³ Motz and Cariani,³⁰ Liebreich,²² and Harris¹⁶ reported cases and supported von Brunn, Aschoff and Lubarsch in their conclusions.

Herxheimer¹⁷ reported two cases of ureteritis and one case of cystitis cystica. He examined twenty-nine cases without gross cysts, making serial sections from selected situations in three. Von Brunn's cell nests or buds were found in eleven of the twenty-nine cases. Glandular elements were not found. Nests were not present when the subject was less than 2 years of age. In one case, a child aged 8 years, von Brunn's cell nests were found. No parasites were found in the cystic contents and the reaction for mucus was negative. He concluded that von Brunn's cell nests are the origin of the cysts, that they are not normal but are commonly present at any age, that they vary in number, size and distribution, and that they are the result of the proliferation of the mucous membrane of the urinary tract following an inflammatory process. The elimination of toxic substances is enough to cause this proliferation and explains the cell nests and cysts found by Marckwald in the new-born.

Giani¹³ introduced into the bladder of rabbits by a suprapubic incision gelatin capsules containing bacilli of tuberculosis, for the study of tuberculosis of the urinary tract. In some instances the capsule passed from the urethra while in others it formed the nucleus for salty incrustation. On killing these rabbits he discovered a condition similar to cystitis cystica. Following this, he denuded areas of the bladder in twenty rabbits, using a Volkmann curet through a suprapubic wound. He killed the rabbits at different times from

forty-eight hours to two hundred seventy days afterward. Sections taken in forty-eight hours showed only scattered bits of epithelium remaining in the places curetted; in five days, granulation tissue and beginning proliferation of the remaining epithelium were evident; in ten days, the epithelium was spread out, practically covering the whole area; in fifteen days numerous mitotic figures were seen in the epithelial cells; in twenty-five days, epithelial buds or sprouts were present; in forty-five days, epithelial cell nests as well as the epithelial buds were present; in seventy-five days, the center of the epithelial cell nests stained poorly and in places a lumen was seen; in one hundred days, the cysts were increased in size and some were approaching the surface, and, with a magnifying glass, the cysts were just visible on the surface; in one hundred twenty days, the cysts were diminished in number; in one hundred sixty days, the mucosa was practically normal; in two hundred seventy days, all signs of the condition had disappeared. He concluded that any irritation of the mucous membrane will cause proliferation of the epithelium and the formation of von Brunn's epithelial buds and cell nests, and that after the nests have formed they progress to the point of forming cysts and eventually rupture into the lumen of the urinary tract even in the absence of the original irritation.

Since Giani's experiments, many papers have been written on pyelitis cystica, ureteritis cystica and cystitis cystica, and several cases reported. Notable among these were the papers of Stow,⁴¹ Saltykow,³⁶ Albersloetter,² Augier and Lepoutre,⁴ Hibbs,¹⁸ and Jacobson.¹⁹

More than sixty cases of pyelitis cystica, ureteritis cystica, or cystitis cystica have been reported in the literature; cystitis cystica predominates. In many of these there were only a few cysts in the trigone of the bladder. Table I includes the most pronounced cases reported. The theories as to the cause of these conditions may be summarized as follows: (1) inflammatory closure of glands in the urinary tract; (2) inflammatory closure of crypts of the mucous membrane; (3) parasitic invasion of the urinary tract, and (4) degeneration of central epithelial cells or the secretory action of the epithelial cells of the epithelial cell nests of von Brunn.

There are likewise conflicting theories as to the formation of the epithelial cell nests of von Brunn which may be summarized thus: (1) That they are inflammatory in nature and are formed by down-

TABLE I
Marked Pyelitis, Ureteritis or Cystitis Cystica

Case	Author	Sex	Age, years	Urinary history	Urinary findings
1	Morgagni	M	60	Burning	Slight bilateral hydronephrosis and hydro-ureter. Cysts of renal pelvis and ureter (bilateral).
2	Morgagni	M	old man	Difficulty 12 years	Bilateral hydronephrosis and hydro-ureter. Right ureteral calculus; cysts of right ureter.
3	Litten	F	75		Right hydronephrosis and hydro-ureter. Right ureteral calculus; cysts of right ureter.
4	Lubarsch	M	75		Purulent pyelitis and cystitis. Dilated ureters. Vesical calculi. Cysts both ureters.
5	Lubarsch	F	78		Bilateral pyelonephritis. Ureters dilated and covered with cysts.
6	Ajutolo	F	40		Duplication of left kidney, pelvis and ureter. Lower pelvis and ureter dilated and showing cysts.
7	Ebstein	M	48	Suppuration of urinary passages; hematuria	Chronic pyelonephritis, ureteritis and cystitis. Left ureteral calculus. Cysts left renal pelvis and ureter.
8	Kahlden	F	66		Chronic interstitial nephritis with recent parenchymatous nephritis and cystitis. Cysts of upper right ureter.
9	Kahlden	M	67		Carcinoma of bladder. Cysts both ureters and renal pelvis.
10	Albersloetter	F	60		Bilateral hydronephrosis. Diverticulum of bladder with cystitis. Cysts both ureters.
11	Albersloetter	F	71		Vesical mucosa swollen and red; small blisters around neck. Cysts both ureters, especially right.
12	Liebreich	M	53	Hematuria	Ureters slightly dilated. Bladder dilated and hypertrophied. Cysts both ureters.
13	Herxheimer	M	25		Trabeculated and hypertrophied bladder. Bilateral slight hydronephrosis. Cysts both ureters.
14	Herxheimer	F	69		Pyelitis and ureteritis with necrosis of the renal papillae. Cysts of renal pelvis and ureters.
15	Augier and Lepoutre	F	86		Pyelonephritis and ureteritis. Atrophic kidneys. Left hydronephrosis and hydro-ureter; cysts left ureter.

Case	Author	Sex	Age, years	Urinary history	Urinary findings
16	Motz and Cariani	M	71		Cystitis. Cysts of trigone and fundus of bladder.
17	Motz and Cariani	M	63		Carcinoma of penis. Duplication of left ureter. Cysts of left ureters.
18	Stow	F	40		Chronic cystitis. Pyelonephritis. Complete duplication of pelvis and ureter on both sides. Numerous cysts of all four ureters.
19	Silcock	M	20		Left kidney removed. Right ureteral calculus. Cysts of bladder.
20	Voelcker	M	73		Chronic interstitial nephritis with formation of numerous cysts. Cysts left renal pelvis and ureter.
21	Hibbs	M	25		Calculus in bladder. Bilateral hydronephrosis and hydro-ureter. Cysts both pelves and ureters.
22	Harris	F	58		Death following operation for right renal calculus. Inflammation of right ureter and bladder with dilatation of ureter. Cysts right ureter.
23	Harris	M	54		Died following operation for right renal calculus. Duplication of left pelvis and ureter with calculus in upper pelvis. Cysts in ureter to upper pelvis.
24	Clark	F			Left hydronephrosis. Cysts of upper left ureter, pelvis and bladder.
25	Eve	F	51	Frequency, hematuria	Cysts both pelves and ureters.
26	Stoerk	F	57		Chronic cystitis. Cysts of both ureters.
27	Stoerk	F	60		Pyelitis, ureteritis, and hemorrhagic cystitis. Right renal stone. Cysts in ureter and trigone of bladder.
28	Jacobson	M	83	Operation; prostatic trouble 8 years before death	Duplication left pelvis and ureters to middle third. Atrophic kidneys with evidence of old pyelonephritis. Cysts both ureters and pelves on both sides.
29	Jacobson	M	74	Nocturia 15 years	Cysts of bladder.
30	Jacobson	M	66	Prostatectomy 2 years before death	Old pyelonephritis and cystitis. Cysts of ureters and pelves.

ward proliferation of the epithelium, or proliferation of the connective tissue upward between the epithelial nests to cut off islands of epithelial cells, and (2) that they are at times normally found in the urinary tract.

CASES STUDIED

One hundred twenty-five cases were observed practically consecutively at necropsy and three cases recognized macroscopically as pyelitis cystica, ureteritis cystica and cystitis cystica.

In 100 of the 125 cases, sections were taken from each renal pelvis and ureter, and from the fundus and trigone of the urinary bladder. In the remaining twenty-five cases, the renal pelvis and ureters were removed intact and mounted. Sections were taken at seven different levels, namely, renal pelvis, ureteropelvic juncture, upper, middle and lower thirds of the ureter, the fundus and trigone of the bladder.

The average age of the 125 subjects was about 44½ years, the oldest being 88 years and the youngest a fetus born in the fifth month of pregnancy.

Macroscopically, cysts were not found in the group of 125 cases. Microscopically, the so-called epithelial buds and cell nests of von Brunn or cysts were found in varying numbers and situations in 108 of the 125 cases (86.4 per cent). In Table II is a brief summary of seventeen cases in which epithelial buds, cell nests or cysts were not found.

Inflammation as judged by lymphocytic infiltration, proliferation of fibrous connective tissue, and formation of new capillaries was found in 63 (58.25 per cent) of the 108 cases showing epithelial buds, cell nests or cysts, and in four (23.6 per cent) of the cases in which no evidence of epithelial buds, cell nests or cysts were found.

In ten of the twenty-five cases in which sections were taken at seven different levels, epithelial buds, nests or cysts were found in the renal pelvis in ten cases, in the upper ureter in fifteen cases, in the lower ureter in seven cases and in the bladder in twelve cases. This confirms Aschoff's opinion that they are most common in the renal pelvis, upper portion of the ureter and bladder.

Serial sections were made at different levels in fifteen cases to establish, if possible, the presence of glands or uniform connections between the epithelial cell nests and the surface epithelium, or the presence of ducts connecting the cystic formation with the lumen of the renal pelvis, ureter or bladder. The epithelial sprouts or buds,

TABLE II

Seventeen Cases: No Epithelial Buds, Nests or Cysts

Case	Sex	Age	Inflammation	Cause of death
1	M	Full term	Absent	Still birth.
2	M	One-half hour	Absent	Prematurity (sixth month).
3	M	Eighth month	Absent	Still birth.
4	F	Six days	Absent	Prematurity (sixth month).
5	F	Three months	Absent	Cardiac anomaly.
6	F	Years 4	In bladder	Enucleation of right eye for neuroblastoma of the retina. Terminal bronchopneumonia.
7	F	5	Absent	Repair of right diaphragmatic hernia.
8	M	12	Absent	Glioma of the cerebellum with internal hydrocephalus.
9	F	13	Absent	Generalized peritonitis from a ruptured appendix.
10	M	18	Absent	Anterior poliomyelitis.
11	M	26	Wall infiltrated with myelocytes and other cells	Myelogenous leukemia.
12	M	38	Absent	Exploration of brain tumor. Cystic glioma of right cerebrum.
13	M	42	Slight throughout	Cholecystectomy for subacute cholecystitis with cholelithiasis. Edema of brain.
14	F	53	Absent	Cholecystectomy and appendectomy for chronic cholecystitis and appendicitis. Generalized peritonitis.
15	F	56	Subacute cystitis	Carcinoma of the ovaries with metastasis to the omentum, right ureter, and supraclavicular lymph nodes.
16	F	61	Absent	Carcinoma of the rectum with perforation and generalized peritonitis.
17	M	77	Chronic throughout	Carcinoma of the pancreas with metastasis to the liver and pleura.

as their name implies, are connected with the surface epithelium. A duct-like connection was not seen between the cystic structure and the lumen of the renal pelvis, ureter or bladder. In all cases, cysts can be seen opening on the surface. They have ruptured spontaneously or possibly in sectioning; but when the latter occurs one sees the cysts close to the surface and a good portion of the cystic envelope has disappeared.

Glands were not found in the urinary tract in the routine slides or in the serial sections, except in the trigone, where they were close to the internal sphincter of the urethra. These glands have been thoroughly studied and described by Albarran,¹ Lowsley²⁵ and others. As a rule, they are straight tubular structures lined by a double layer of cuboidal cells. Occasionally, one sees a nodule in the trigone of the bladder which somewhat resembles a cyst but which on section proves to be an encapsulated adenoma.

Egli, Litten, Hamburger and others undoubtedly saw formations which resembled glands, that is, cystic formations lined by several layers of cells, the inner layer being columnar and arranged axially.

Stoerk and Zuckerkandl⁴⁰ did not call these glands, but believed the lumen to be produced by the secretory action of the cells. Dogiel⁹ states that the surface epithelium of the urinary tract is secretory and in this Stoerk and Zuckerkandl support him in part. They state that the surface epithelium is secretory in some instances when there is increased vascularity due to inflammation, but that, whatever the secretion is, it is not mucous in character and may be only a product of degenerated epithelium. In secretions of the urinary tract, taken by catheterization and cystoscopy, they were never able to demonstrate a mucous reaction, whereas in the cystic contents there was a mucin reaction on a few occasions.

Occasionally, in these cases, I have found the surface epithelium to resemble secretory epithelium, it being columnar in type and the nuclei situated basally. In numerous specimens of the fresh cystic material, specially fixed and stained with mucin stains, I was unable to demonstrate a mucin reaction in any instance.

That the crypts of the mucous membrane may occasionally become closed off by proliferation of the fibrous tissue is easily conceivable when one sees the close proximity between the walls at the mouths of the crypts and the marked proliferation of fibrous tissue that takes place in many cases.

Those advancing the theory that these cysts are caused by parasites did so merely on the histologic appearance of certain ovoid bodies found in the cystic contents, which they maintained resembled certain coccidia. They were unable to culture these parasitic forms and, in the examination of the fresh contents of the cysts, no motile forms were found. These forms were never seen in the surrounding epithelium nor was it possible to produce the disease in animals from the contents of the cyst. Gilchrist,¹⁴ in an exhaustive study of the so-called psorospermatic diseases of the skin, has shown that degenerating epithelium gives rise to particles that simulate and have been called psorosperms. It has been established by Giani that cysts form from the epithelial cell nests of von Brunn. The method of formation of these nests is not clear. Von Brunn, Aschoff, Lubarsch, Stoerk and many others have considered them inflammatory in nature, while Giani has proved experimentally that they form as the result of inflammation. Marckwald has stated that they are occasionally normal constituents and reports a case in which they were present in a new-born child, although Herxheimer explains this by the excretion of toxic substances in the urine. Giani also states that, once the epithelial cell nests have formed, the cell nests will go on to cystic formation and eventually disappear even in the absence of the original causative factor and all signs of inflammation.

In sixty-three (58.25 per cent) of the 108 cases in which epithelial buds, cell nests or cysts were present, inflammation was manifested by lymphocytic infiltration or fibrous tissue proliferation, while in only four (23.5 per cent) of the seventeen cases remaining was there evidence of inflammation.

Fifteen of the 125 patients were under 20 years of age. In five of these fifteen cases epithelial buds, cell nests or cysts were found. Table III gives a brief summary of these cases.

It has been stated that these cysts, although inflammatory in nature, may be formed by two separate reactions of the tissue to inflammation, that is, by the proliferation of the epithelium and by the proliferation of the fibrous connective tissue. That they are formed in most cases by a combination of the two seems the most probable deduction, as they go hand in hand in inflammation of the urinary tract.

The variation in the size of the cysts is explainable on a purely

mechanical basis; that is, the size of the cyst varies with the amount and strength of the surrounding fibrous envelope, which depends, as in other conditions, on the severity and chronicity of the inflammation.

Jacobson proposed another factor in the formation of these nests: with old age and the consequent arteriosclerotic changes, the muscular walls are undernourished, and consequent muscular atrophy and atony produce excessive infolding of the mucosa, which is more

TABLE III

Five Cases Showing Epithelial Buds, Nests or Cysts (patients under twenty years of age)

Case	Sex	Age, years	Inflammation	Cause of death
1	M	5	Present	Chronic otitis media with purulent meningitis; internal hydrocephalus.
2	F	15	Present	Exophthalmic goiter. Pyelonephritis with multiple cortical abscesses.
3	M	16	Present	Cellulitis of face and neck from a furuncle. Hemorrhagic bronchopneumonia. Thrombosis left jugular vein. Multiple abscesses left kidney.
4	F	17	Present	Extensive superficial burns. Extreme emaciation. Calcium deposits both kidneys.
5	M	18	Absent	Sarcoma left leg with metastasis to lungs, liver, small intestine, and right kidney.

easily cut off into nests by proliferation of fibrous connective tissue. The formation of nests in the absence of arteriosclerotic changes he attributes to the inflammatory closure of crypts. This theory does not hold, since, in the absence of arteriosclerosis, definite epithelial budding and nest formation have been demonstrated.

In these cases the mucosa of the urinary tract reacted in four separate ways or in any combination of these four: (1) by desquamation of its epithelium, as seen in acute inflammatory processes; (2) by a papillary overgrowth or, as it is sometimes called, villous cystitis; (3) by proliferation of the lower layer of the epithelium with the formation of epithelial buds or sprouts; and (4) by cornification of the epithelium or, as it is commonly known, leukoplakia.

ILLUSTRATIVE CASES

CASE 1. A woman, aged 46, complained of a mass in the left lower portion of the abdomen. Eight years before, a ventral hernia had been repaired, and one year following this an operation was performed for intestinal adhesions. During the last two years, she had had attacks of crampy abdominal pain, which occurred two to three times a week and were associated with severe constipation. There was no history of urinary symptoms.

There was an incarcerated left inguinal hernia and a small umbilical hernia. The urine contained ten pus cells to a high power field. At operation these hernias were repaired and at that time cholecystitis and cholelithiasis were found. Fourteen days later, cholecystectomy was performed. Three days afterward the patient died.

At necropsy a left-sided subdiaphragmatic abscess and myocarditis were found, as well as acute nephritis implanted on chronic nephritis. The left renal pelvis and ureter on both sides showed isolated, bleb-like cysts up to 4 mm. in diameter, which contained a slightly brownish, gelatinous or sago-like substance (Fig. 1). Cysts were not found in the bladder.

CASE 2. A woman, aged 76, complained of dizziness associated with numbness of the right forearm and hand for the previous six months. The peripheral arteries showed marked sclerosis. The cardiac dulness was increased. The systolic blood pressure was 194 and the diastolic 86. There was definite weakness of the muscles of the right hand and forearm. The specific gravity of the urine was 1.014; it was alkaline and gave a definite reaction for albumin. Microscopically, there were from one to fifteen pus cells in each high power field. The patient died four days after admission.

At necropsy acute nephritis superimposed on chronic nephritis, myocarditis with coronary sclerosis, chronic vegetative aortic and mitral endocarditis, general arteriosclerosis, chronic catarrhal cholecystitis and cholelithiasis and left nephrolithiasis were found. The left renal pelvis and ureter showed several translucent cyst-like formations, some of which appeared pedunculated and contained a yellowish gelatinous material (Fig. 2).

CASE 3. A man, aged 64, complained of a general breakdown one year previously. At the time of this breakdown he was considerably

improved by treatment in a hospital for a month. The breakdown was characterized by dyspnea and edema of the lower extremities, both of which returned after dismissal from the hospital. In the year there had been a gradual increase in the severity of these symptoms and mental failure had progressed.

The patient was emaciated and dyspneic and came in a wheel-chair. The right side of his face was flaccid and the tongue protruded to the left. The peripheral arteries were markedly sclerosed. The systolic blood pressure was 170 and the diastolic 110. The apex beat was diffuse and considerably to the left; a blowing systolic murmur was heard best over the pulmonic area. There was marked edema of the lower extremities. The urine was acid and its specific gravity 1.007; there was marked reaction for albumin. There were numerous granular and hyaline casts and from thirty to fifty pus cells in each high power field. The blood urea was 456 mg. for each 100 cc. Death ensued eighteen days after admission.

Necropsy showed marked generalized arteriosclerosis, cardiac hypertrophy and dilatation, atrophy and scarring of the kidneys, and multiple cortical cysts following old healed pyelonephritis. Both the renal pelvis and ureter on each side showed isolated cyst-like structures. Chronic cystitis was present, and the trigone showed multiple cyst-like formations (Fig. 3).

DISCUSSION OF CASES

Microscopic sections in these cases showed numerous epithelial cell nests and cysts in all stages of development associated with a diffuse inflammatory process. The lumen was filled with a colloid-like material in which red blood corpuscles and degenerated epithelial cells were seen. The lining of these macroscopic cysts, as a rule, consisted of a single or double layer of flat epithelial cells, resembling somewhat endothelial cells, surrounded by a dense fibrous tissue envelope.

It is rather hard to understand why epithelial cell nests and microscopic cysts are common while macroscopic cysts are so seldom found at necropsy. In a cystoscopic experience of any extent, it is rather common to find cysts of the mucosa of the bladder in cases of long-standing inflammatory disease. In the examination of the records of 190 cases of bilateral pyelonephritis, cystitis cystica was found in

thirty-three (17.4 per cent). The cysts are usually found in the trigone and base of the bladder but are occasionally diffusely scattered over the remainder of the mucosa.

This seemingly conflicting evidence is probably in part due to the fact that, unless carefully searched for, the smaller cysts are easily overlooked, particularly in the presence of marked inflammatory reaction.

CONCLUSIONS

1. Glandular structures are not normally found in the urinary tract above the trigone of the bladder.
2. Epithelial buds, cell nests, and cysts are not normally found in the urinary tract.
3. Cysts of the urinary tract are formed from the epithelial nests of von Brunn.
4. The epithelial buds and cell nests of von Brunn are inflammatory in nature.
5. Microscopically, epithelial buds, cell nests and cysts are present in the majority of persons more than 20 years of age.
6. This study does not reveal evidence to support the secretory theory of the formation of these cysts.
7. The cysts are formed by degeneration of the central cells of the epithelial cell nests.

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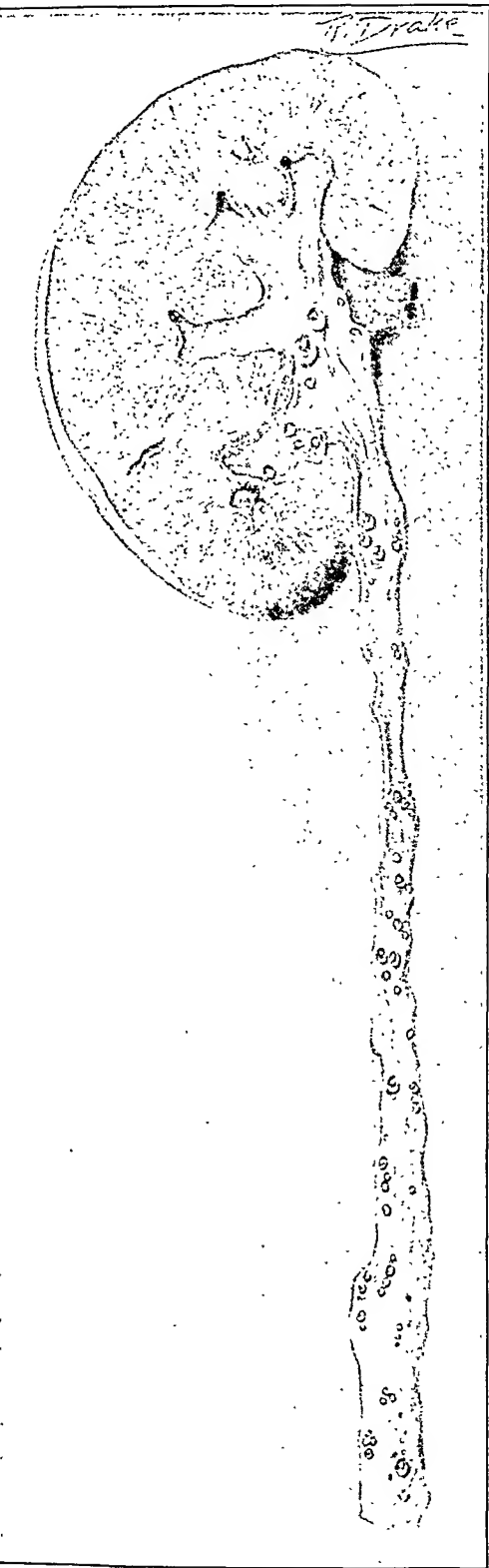
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DESCRIPTION OF PLATE

PLATE 15

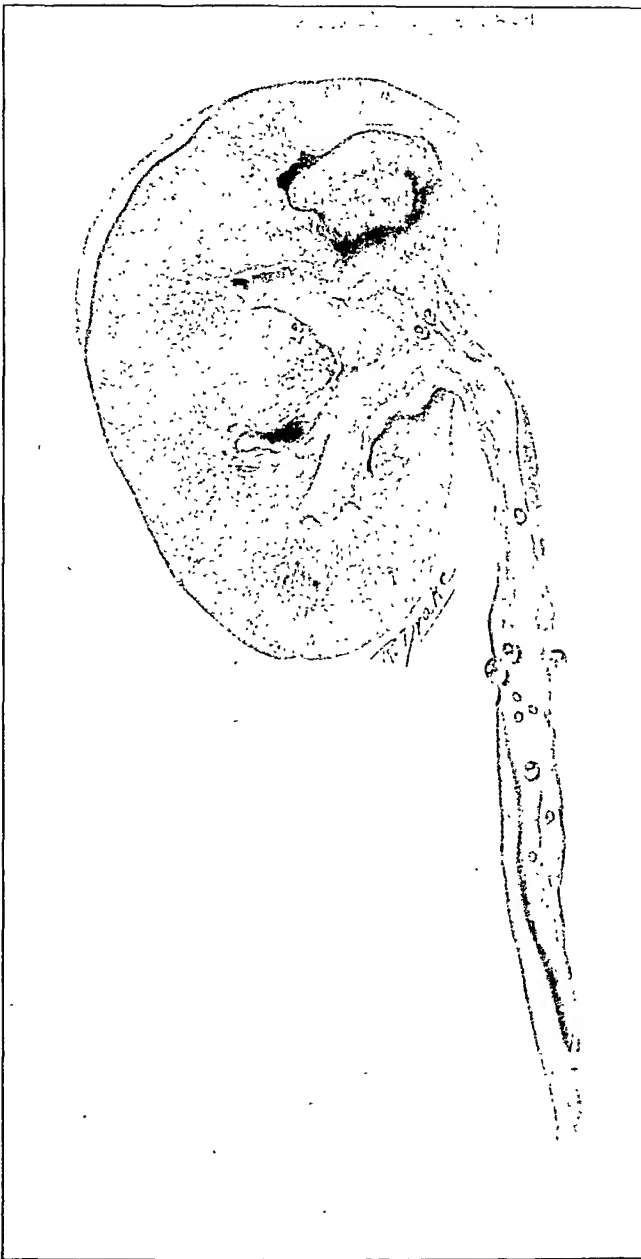
FIG. 1. Case 1. Left kidney and ureter. Cysts of the renal pelvis and ureter.

FIG. 2. Case 2. Left kidney and upper ureter. Calculus in upper calyx and cysts in the renal pelvis and ureter.



1

Morse

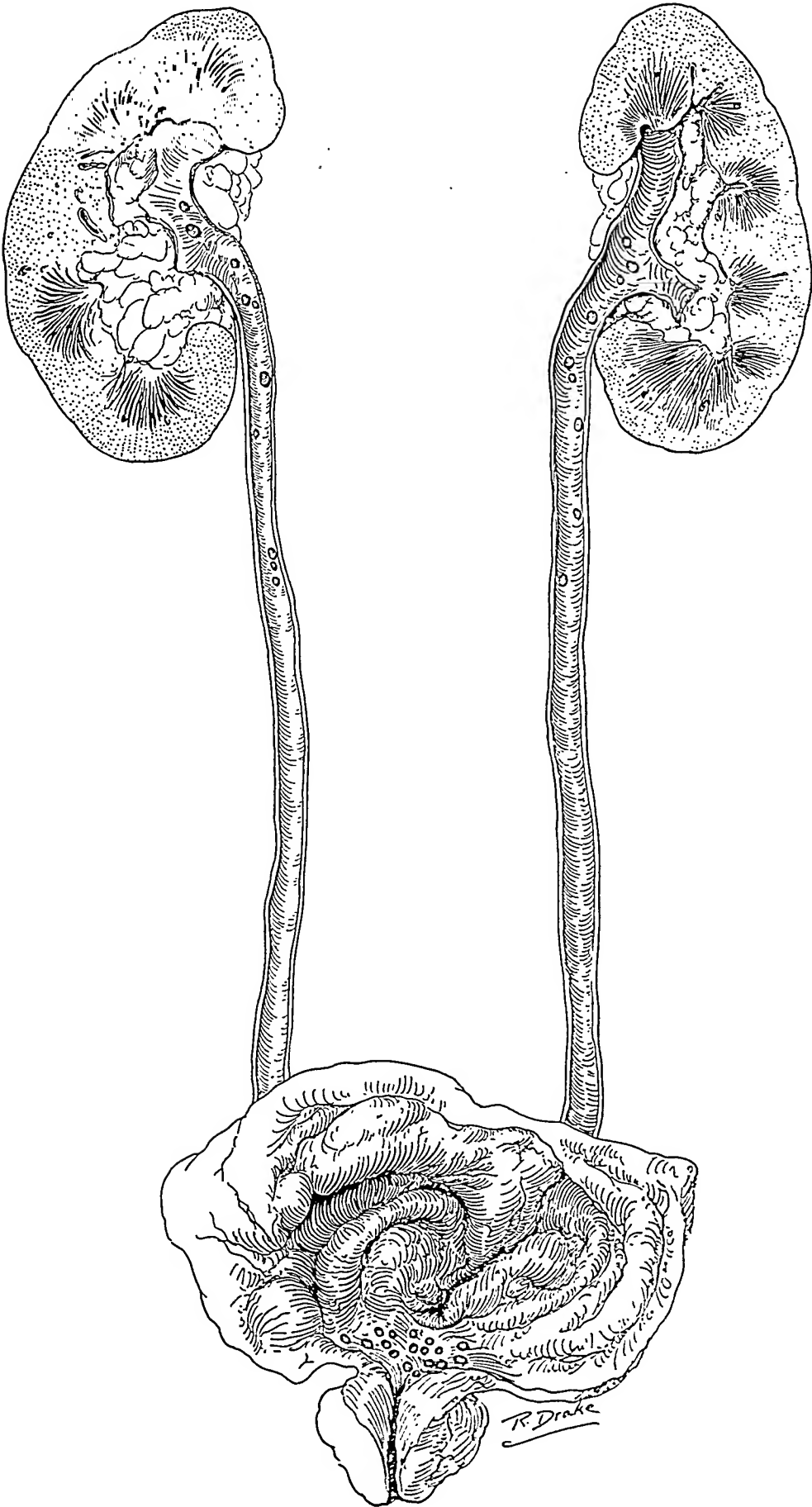


2

Pyelitis, Ureteritis and Cystitis Cystica

PLATE 16

FIG. 3. Case 3. Kidneys, ureter and bladder; cysts of the renal pelvis, ureters and trigone of the bladder.



A MALIGNANT TUMOR SIMULATING BONE MARROW *

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Heterotopia of the bone marrow is not an extremely rare lesion and usually is represented by small masses of myeloid tissue. Saleeby¹ has recently recorded a case found at necropsy and has briefly summarized the literature. Development of bone marrow in metaplastic bone is not an unusual finding. Thus it is encountered in old inflammatory processes, such as in the wall of an appendix, the site of a previous appendicitis, or in the walls of sclerotic arteries, in slowly-growing tumors and numerous other places.

Freudenstein² presents a careful study of the problem of the development of bone marrow in heterotopic bone and considers three theories as to its origin:

- (1) Adventitious cells can produce the various cell forms of the marrow, even including the red blood cells.
- (2) The elements of the normal bone marrow can circulate in the blood and be deposited in the regions of bone formation.
- (3) In engorged capillaries or veins lymphocytes enlarge and become changed to typical large proliferating lymphocytes. From these develop intra- and extravascular myelocytes, red blood cells and megakaryocytes.

Heterotopic bone marrow has been not infrequently reported in the pelvis of the kidney or in the adrenals. These cases have usually been associated with myelogenous leukemia or the so-called splenic anemia.^{3, 4, 5} Mieremet⁶ reports a striking tumor, found in the adrenal of a man dying of carcinoma of the esophagus, showing the histologic structure of fat cells separating myelocytes, myeloblasts, erythrocytes, erythroblasts, polymorphonuclear leucocytes and megakaryocytes. Heterotopic marrow may occur also in connection with endotheliomas. Thus in an actively growing endothelial tumor of the liver, Fischer⁷ found islands of blood-forming tissue containing nucleated blood cells and myelocytes.

* Received for publication August 26, 1927.

In none of these cases, however, have the masses of heterotopic marrow presented other than a chance association with the cause of death. The following case presents such unique features that it seems worth while to place it on record.

REPORT OF CASE *

Clinical History: J. J., 42 years of age. In November 1925 patient, an unmarried white female, was run down and in a poor condition. On examination sugar was found in her urine. With 45 units of insulin daily, she became and remained sugar free although the blood sugar was not reduced below 250 mg. Her spleen was enlarged and the red cell count varied in the neighborhood of 2,000,000, with many immature forms, whereas her white cell count ranged about 8,000. While getting out of bed she struck her nose, causing uncontrollable hemorrhage which resulted in her death.

NECROPSY REPORT

H-26-166. *Body:* The body is that of a well developed and extremely obese white woman. External examination is negative except for bloody fluid in her nose and a small firm mass adherent to the outer border of the left sixth rib near the costochondral junction.

Peritoneal Cavity: The peritoneal cavity contains a small amount of thin blood-tinged fluid. The mesenteric lymph nodes are not enlarged.

Pleural Cavities: There is about one liter of thin blood-tinged fluid in each pleural cavity. On the inner surface of the chest wall are several smooth, rounded swellings firmly adherent to the periosteum of the ribs. These measure about $3 \times 1.5 \times 1.5$ cm. On section they are pale pink, friable, soft and of uniform appearance and connected with the substernal mass about to be described. The under surface of the sternum, particularly at its upper end, and the adjacent portions of the costal cartilages are covered over with a firm, very dark red, apparently hemorrhagic mass 1 to 2 mm. thick at the edges and gradually thickening toward the center where it reaches a depth of 1 cm.

Heart and Pericardial Cavity: Negative.

Lungs: Essentially negative.

Spleen: Weight 1500 gm. There are a number of fibrous adhesions to the parietal peritoneum. On section the organ is very soft

* This case was summarized in the Cabot case records, Boston M. & S. J., 1927, cxcvi, 657.

and purplish red in color. All the normal markings are obscured and the pulp drips from the cut surface.

Liver: Weight 5400 gm. Capsule is smooth and the liver is pale, of an opaque yellowish gray color. On section it cuts readily and the markings are fairly distinct with slight exaggeration of the usual lobular appearance. The cut surface and the knife are greasy.

Gall Bladder: The gall bladder contains two rounded soft, yellow-brown stones 0.5 cm. in diameter.

Gastro Intestinal Tract: Negative.

Pancreas: Negative.

Kidneys: The left kidney weighs 175 gm. It is adherent to its bed but the capsule strips fairly easily, leaving a smooth surface. The ureter is seen to be greatly thickened and a red fleshy substance is present in the hilum of the kidney. When sectioned a red fleshy mass is revealed between the pelvis and the kidney tissue, ranging from 0.3 to 1.5 cm. in thickness. At no point has it ulcerated through into the pelvis and it is apparently not invading the kidney tissue, but rather insinuating itself between the parenchyma and the epithelium of the pelvis. This mass is extending beneath the pelvic epithelium and its edge is distinctly hemorrhagic. The cortex is 1.8 cm. thick. The glomeruli are visible as minute specks. The pyramids are not particularly well marked. The same description is true for the right kidney with the exception that it weighs 225 gm., and that the tumor mass is slightly more extensive. Both ureters are encased in a firm reddish mass continuous with that described beneath the pelvic epithelium, and extending the entire length of the ureters into the reddish mass which becomes merged with the pelvic musculature. There is no obstruction to the lumen of either ureter and so far as can be determined no ulceration into their lumina.

Adrenals: Negative.

Bladder: Negative.

Genitalia: Essentially negative.

Pelvis: The entire pelvic musculature including the psoas, iliacus and the levator ani is merged into the firm reddish mass continuous with the growth described about the ureters. It is entirely retroperitoneal. On section the tissue is dark red but contains rather sharply defined pale foci varying in size and shape. The consistence is apparently the same throughout. There is no continuity with or invasion of the rectum or the other pelvic organs.

Lymph Nodes: The retroperitoneal lymph nodes from the pelvic floor to the diaphragm are enlarged. Some are soft, pale pink and friable while others are very firm and dark red. There is practically a continuous cord of tumor tissue connecting the retroperitoneal lymph nodes and extending into the mediastinum. The mediastinal lymph nodes present a similarly varied appearance and are involved in the same process as those below the diaphragm. There is one very firm lymph node about 1 cm. in diameter beside the head of the pancreas. On section this node shows marked carbon pigmentation.

Aorta: There are a few atheromatous plaques in the abdominal portion of the aorta, one of which has become calcified.

Bone Marrow: The bone marrow of the ribs is a pale pink, that of the bodies of the vertebrae is somewhat darker, but shows no apparent variation from the normal.

MICROSCOPIC EXAMINATION

Heart: Essentially negative.

Lungs: Pleura slightly thickened and, in one small focus, contains clusters of cells, some of which resemble lymphocytes and plasma cells, others endothelial leucocytes, and others eosinophiles and eosinophilic myelocytes. Lung capillaries are congested and a few megakaryocytes are present in them.

Spleen: But few lymph nodules can be distinguished. Marked congestion. Numerous clusters of large cells with vesicular nuclei and relatively small amounts of pale cytoplasm are scattered through the pulp together with nucleated red cells in various stages of development, eosinophiles and eosinophilic myelocytes (Fig. 1).

Pancreas: Lobules are separated by fat tissue. Slight increase in interacinar connective tissue. Islands are few but most appear normal though some contain considerable amounts of hyaline material. The peripancreatic fat is infiltrated by cells with large clear nuclei similar to those described above, by nucleated red cells and by neutrophilic and eosinophilic myelocytes.

Liver: Many of the liver cells contain large vacuoles. The nuclei of a considerable number are swollen and clear with relatively small masses of chromatin. The portal regions are infiltrated by cells with but little cytoplasm whose large nuclei contain scattered chromatin granules, by nucleated and a few non-nucleated red cells and by eosinophilic myelocytes. An occasional megakaryocyte is present.

Small islands of similar cells occur in many of the sinusoids. The nuclei of the endothelial cells lining the sinusoids are rounded and prominent. The histologic picture is strikingly like that seen in the liver of myelogenous leukemia.

Kidney: Slight postmortem change. Glomeruli negative. Colloid degeneration of tubular epithelium. There is some vacuolization of the epithelium of Henle's loops due to glycogenic infiltration resulting from diabetes. The region of the pelvis contains a large mass of hematopoietic tissue which under low power resembles hyperplastic marrow, showing scattered fat cells throughout. One small spicule of bone is present in the pelvic region. Under high power sinusoids can be made out. There are numerous red blood cells in all stages of development. Eosinophilic myelocytes and megakaryocytes (Fig. 2) are also present. The most common nucleated cell is similar to that already described in pleura, spleen and liver. This cell appears to be the hemocytoblast. The hematopoietic tissue is seen to penetrate the kidney substance for some distance, usually in close relation to the larger blood vessels.

Adrenal: There is a considerable amount of hematopoietic tissue in the surrounding fat.

Ureter: The wall of the ureter is infiltrated and greatly thickened by hematopoietic tissue similar to that described in the kidney (Fig. 3). The mucosa and muscularis mucosae are not involved.

Pelvic Musculature: Here and there strands of fibrous tissue and of muscle fibers, some of which are necrotic, traverse a mass of hematopoietic tissue. In one section a portion of the lumbar plexus is present and the perineurium is penetrated by the bloodforming tissue in several places.

Uterus: Cells with large vesicular nuclei similar to those described in the liver, spleen and kidney, surround the arteries and penetrate the musculature to some extent. The mucosa is atrophic.

Lymph Nodes: The sinuses are infiltrated by rather large cells similar to those described elsewhere.

Substernal Mass: This same type of large cell together with great numbers of red blood cells infiltrate the connective tissue. The appearance is almost hemorrhagic in places. In other regions among the more primitive cells are scattered eosinophilic and neutrophilic myelocytes, nucleated red cells, and megakaryocytes. The capillaries are large and somewhat tortuous.

Bone Marrow: The marrow is not remarkable and very few of the primitive type of cell, so common in the abnormal marrow-like tissue, are found. Unfortunately, fixation is extremely poor.

DISCUSSION

The exact nature of this tumor is uncertain. It obviously is a tumor and not a concomitant phenomenon of a leukemia or other disease involving the hematopoietic system. The histologic structure and the clinical history would lead to the belief that these cells did not gain access to the circulation in any considerable amount and that the anemia present was secondary anemia such as might be associated with any advanced malignant tumor. The widespread distribution of the lesions suggests a very malignant type. It is striking that all parts of the tumor, even the mass beneath the sternum, are continuous with one another.

Just what proportion of the symptoms noted in her illness of four months is referable to her co-existent diabetes is uncertain. However, the fact that she did not show much clinical improvement with insulin treatment leads to the belief that most of her symptoms were due to the growth of the tumor. The large proportion of the reported cases of heterotopic marrow which are found in the kidney pelvis suggests that here may be the primary site of this tumor.

Curiously enough, Jordan and Speidel⁸ bring out phylogenetic evidence as to the myeloid function of the kidney. They state that in the tadpole the kidney is the chief lymphomyeloid organ. The hematopoietic activity is centered in the intertubular connective tissue (mesonephros). There are transition forms present between lymphocytes, red blood cells and granulocytes. In the adult frog the mesonephros produces chiefly granulocytes, and those in small numbers, while the spleen has become the important hematopoietic organ. Whether there is any relationship between this condition in the frog and the tendency of heterotopic marrow to occur in the kidney of man is of course purely speculative, but nevertheless worthy of passing mention. In the light of this it is quite possible that the tumor was primary in the pelves of the kidneys and extended down the ureters, spreading by way of the lymphatic system through the retroperitoneal and mediastinal tissues and ultimately reaching the spleen and liver. When heterotopic marrow occurs in a kidney it is usually found in both.

Another possible source, however, is the retroperitoneal fat tissue. Petri⁹ in an examination of the retroperitoneal adipose tissue of forty adults, most of whom had died from infection or sepsis, but including also some cases of pernicious anemia, leukemia and tumors, found thirty-one to show small hemorrhagic lesions varying in size from a pinhead to a bean and having the appearance of hemorrhagic lymph nodes. Microscopically the islands were seen to be composed of relatively undifferentiated cells among which could be seen evidence of hematopoiesis of both the lymphocytic and the myelocytic series. He considers this adipose tissue as mesenchymal reticulo-endothelial tissue which retains the embryonic potentialities of hematopoiesis. He believes the change to be induced in the adipose tissue by the action of toxic or bacterial stimuli. It is certainly true in this case that the retroperitoneal adipose tissue would more nearly represent the center of the neoplastic tissue than would the kidney pelves.

As to the nature of the tumor growth itself, it is very difficult to determine. Judging from the tendency to take on the typical morphology of bone marrow including fat cells and even (in the case of the hilum of the kidney) spicules of bone, it might be considered a myeloma of the mixed cell type. The predominant cell appears to be the hemocytoblast of Maximow although it is very difficult to identify positively. The bulk of the tumor is made up of relatively large cells with large nuclei, poor in chromatin, and a relatively small amount of cytoplasm free from granules which suggests the hemocytoblast or *Stammzell*. The other cells present, namely, erythroblasts, erythrocytes, megakaryocytes and cells of the polymorphonuclear series suggest also that a very primitive cell form makes up the bulk of the tumor and that this primitive cell is differentiating to the more specialized cell types in the growth process. There are certain cells in the tumor strongly suggesting lymphocytes. Unfortunately, the length of time postmortem (ten hours) has interfered with satisfactory oxidase reactions so that morphology alone can be relied upon. Should these cells be lymphocytes this tumor would provide evidence for the monophyletic origin of the blood cells. For lack of a better term the name hemocytoblastoma is suggested for this tumor.

SUMMARY

1. A case of an extensive tumor resembling bone marrow, apparently primary either in the pelves of the kidneys or the retroperitoneal fat tissue is reported in a woman 42 years of age without evidence of leukemic involvement.

2. The possible sources of origin and the nature of this tumor are considered.

3. The name hemocytoblastoma is proposed.

The writer wishes to acknowledge the interest of Dr. William B. Breed under whose care this patient was, and also his indebtedness to the House of the Good Samaritan where the necropsy was done.

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DESCRIPTION OF PLATE

PLATE 17

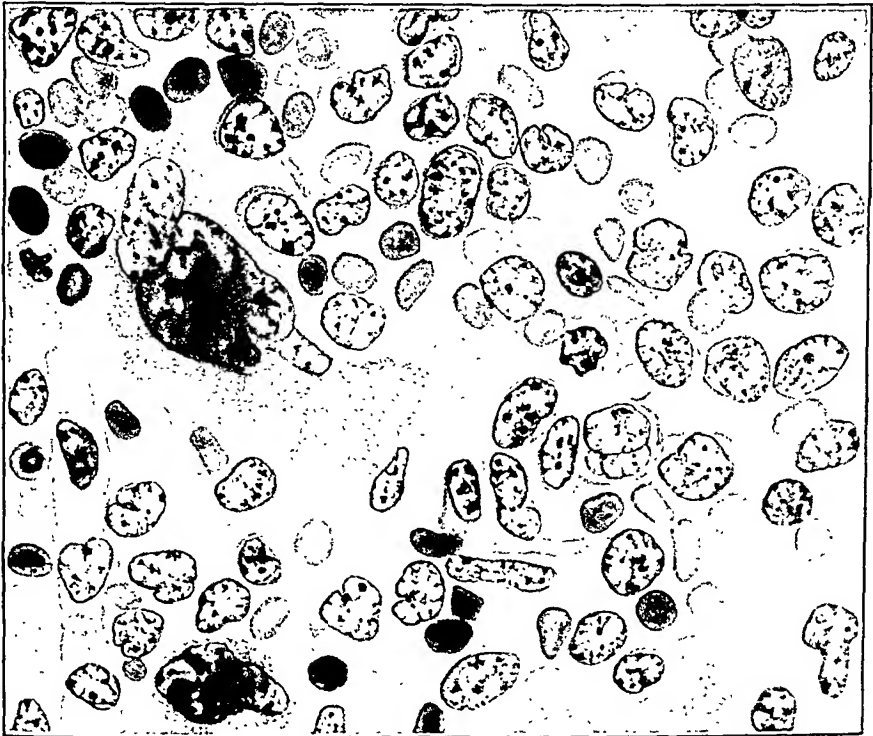
- FIG. 1. Spleen. Note mitosis in erythroblast. $\times 1250$.
 FIG. 2. Kidney. Megakaryocyte. $\times 1250$.
 FIG. 3. Ureter. Drawing showing different types of cells. $\times 1500$.



1



2



3

CONCERNING ECTOPIC CHORIONEPITHELIOMA *

REPORT OF TWO CASES

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Marchand¹ in 1895 in describing neoplasms hitherto known as "decidual" tumors, having clearly shown the relation of the tumor cells to chorionic elements, was the first to designate such structures by the term "chorionepitheliomas." The chorionic and the tumor cells are rather similar morphologically, and furthermore a relationship to pregnancy and especially to hydatidiform mole is recognizable. The tumors described by Marchand all developed in the uterus and gave rise to many and varied metastases, but in 1902, Zagorjanski-Kissel² gathered from the literature reports of nine cases; in eight the tumor appeared primarily in the vaginal wall, and in the other case neoplastic foci were found in the lungs and brain without a primary tumor of the genitalia. In Zagorjanski-Kissel's paper and in subsequent studies the view is more and more clearly expressed that there are instances where chorionepithelioma does not arise at the site of placental attachment but rather in distant foci, probably originating from chorionic cells or villi which have penetrated maternal vessels and have been more or less diffusely deposited. Dunger³ spoke of such instances as examples of "ectopic chorionepitheliomas."

It is well known that chorionic elements may penetrate the maternal circulation. Schmorl⁴ examined the lungs of 158 patients dying at different stages of pregnancy or after delivery, and found chorionic cells in the lung capillaries in eighty per cent of the normal pregnancies. In the lungs of eclamptics, chorionic cells obstructed capillaries, sometimes extensively, in every case examined. During the early period of pregnancy emboli of chorionic origin were found in the lungs in three out of twenty-five cases. Twenty-two cases dying after abortion during the first two months of pregnancy

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showed chorionic cells in the lung capillaries of eight, occurring twice in large numbers. In only three cases were evidences of chorionic cell proliferation in the lung observed, and in view of the lack of examination of the placenta, hydatidiform or chorionepitheliomatous changes could not be excluded. Apparently entire chorionic villi may enter the circulation and reach distant foci only in instances of protracted labor, manual removal of the placenta or ruptured uterus. Schmorl noted large numbers of chorionic cells in the lung capillaries and found evidences of proliferation in two cases of hydatidiform mole and in one of partial mole. In accordance with these findings he emphasizes the possibility that ectopic chorionepithelioma may originate from a previous mole or chorionepitheliomatous proliferation of a placenta completely expelled by labor.

Marchand⁵ and Pick,^{6,7} however, maintain that chorionic cells or villi deposited during normal pregnancy may likewise give rise to chorionepithelioma and that most cases of ectopic chorionepithelioma reveal a history of normal pregnancy, a mole being relatively rare. Attempts to produce chorionepithelioma experimentally in animals (Fraenkel⁸) have failed. This investigator injected ground placenta and mixtures of placenta and ovary subcutaneously, intraperitoneally and intravenously in rabbits and guinea pigs. Some animals were observed over a four-year period but no tumors occurred, the injected material undergoing necrosis or absorption. These experiments have little bearing, however, since certain factors other than the mere presence of chorionic elements in various organs are operative in the genesis of tumors, and, moreover, the animals used are not known to have spontaneous chorionepitheliomas. The opinions of Marchand and Pick receive support from an observation by Walthard.⁹ The latter found a chorionepitheliomatous nodule in the vaginal wall of a patient during the eighth month of her fifth pregnancy. Caesarian section and total hysterectomy were performed and no evidences of either tumor or mole were found in the uterus. Death occurred seven months later with chorionepitheliomatous nodules in lungs, liver and left kidney. The author considers the nodules to be metastases from a primary ectopic chorionepithelioma of the vagina developing along with normal pregnancy. This interpretation is nevertheless questionable because there are observations on record of chorionepitheliomas, both uterine and ectopic, with very long latent periods. Koritschner,¹⁰ for ex-

ample, reported an ectopic chorionepithelioma twenty-two years after the last delivery, and Polano¹¹ reported one thirteen years after parturition. Ries¹² noted in a vein of the uterine wall a fairly well preserved chorionic villus eighteen years postpartum.

Chorionepithelioma may occur where there is no history of pregnancy. It may likewise develop in the testis and morphologically resemble the chorionepithelioma of the female. This tumor may also appear primarily in the ovary without previous pregnancy.

Cases of ovarian chorionepithelioma were first reported by Pick.^{6,7} The first, an ovarian dermoid in a patient 30 years of age, showed in places typical chorionepitheliomatous proliferation; the second was an ovarian teratoma in a 9-year-old girl, and portions of this tumor structurally resembled chorionepithelioma. Since Pick's cases were reported, others have appeared in which the possibility of previous pregnancy can be absolutely excluded. Pick and likewise Schlagenhauer¹³ are of the opinion that embryonic elements are present in such teratomas which give rise to the chorionepitheliomatous proliferation, the tumor then developing like a uterine chorionepithelioma. Risel¹⁴ and Mönckeberg¹⁵ suggest that the proliferation begins in the ectodermal portion of the teratoma itself without the presence or development of embryonic cells. Such tumors should, in their opinion, be classified as ectodermal neoplasms with more or less of the morphologic structure of a chorionepithelioma. This probability is favored by the observations of Schmaus¹⁶ and Michel,¹⁷ each of whom reports one instance of carcinoma of the ovary with areas of tissue resembling chorionepithelioma. Both patients were young girls. The chorionepitheliomatous character in Michel's case was especially prominent in the metastatic nodules. Schmaus did not consider his case to be of teratoid origin but could not absolutely exclude that possibility.

We may apparently divide extra-uterine chorionepitheliomas into two classes, placing in the first class the tumors associated with pregnancy, and in the second, those tumors in which chorionepitheliomatous proliferation develops in a teratoma. A recent paper by Bostroem¹⁸ introduces a new interpretation of the origin and development of chorionepithelioma; and were his suggestion acceptable the above-mentioned classification would lose significance. Although Bostroem's theory is most hypothetical, being based on many suppositions, and although it disagrees with all laws of pa-

thology, nevertheless it may be briefly summarized. According to Bostroem the tumor does not originate from chorionic cells but rather from mesenchymal endothelial cells, the so-called serotinal wandering cells. The author considers these cells undifferentiated germ cells and believes that they are the basis of all formative changes. Irritated by humoral influences these cells react by proliferation and form so-called primary tumor cells which by a process of amitotic division become syncytial cells, the latter by further differentiation giving rise to Langhans cells (Marchand). The Langhans cells may undergo but a single mitotic division, after which no further cell division occurs. Bostroem believes that the differentiated cells can no longer divide and that they die quite rapidly. This explains to him the necroses common in the central portions of the tumor. Marchand's observation of the tendency of syncytial cells to grow in regions adjacent to capillaries and sub-endothelially is also differently interpreted by Bostroem since, according to the latter, the syncytial cells are formed primarily in these localities, new capillaries proliferate in the vicinity of the tumor and new syncytial cells continually form from serotinal wandering cells; the tumor thus growing in a certain sense by apposition. He further has no difficulty in convincing himself that the "metastases" are not products of a primary tumor but are the result of an irritative action on undifferentiated germ cells in other organs, similar to that causing the primary tumor. In this sense "metastases" are "sister tumors." We shall not attempt a discussion of Bostroem's paper. In spite of the omnipresence of his undifferentiated germ cells, these facts remain: (1) The great majority of chorionepitheliomas develop after pregnancy and especially after mole; (2) chorionepitheliomas developing without previous pregnancy are exceedingly rare; (3) morphologically the tumors resemble chorionic elements, and (4) although Bostroem believes there is no connection between tumor and chorionic cells distributed by the blood stream, the observations of Nagy^{19, 20} and of Schmorl do not support his view. Bostroem's humoral exciting agent is very hypothetical although certainly some such possibility may well exist; for example, some authors, in view of a tendency toward lutein cyst formation in these cases, trace the tumor to hormonal influences. Again the plasma of pregnant women dissolves syncytial cells, but this solution is not evident in the plasma of cases of chorionepithelioma. However, Bostroem's whole theory

has no firm basis and does not justify abandonment of our classification of ectopic chorionepitheliomas.

SUMMARY OF THE LITERATURE

A survey of the literature has given us twenty-five cases of ovarian chorionepithelioma; of these, in thirteen there was a history of pregnancy; in seven, pregnancy was probably excluded, and in five, the diagnosis was not established beyond doubt. These cases are tabulated below.

TABLE I
Chorionepitheliomas of the Ovary after Pregnancy

Author	Age in years	Number of deliveries	Last previous delivery or abortion	Localization	Remarks
Seitz ²²	27	5	2 months	ovary	no necropsy uterus examined by curetting
Iwase ²³ (first case)	31	2	3½ months	ovary	
Iwase (second case) ...	43	10	1 year	left ovary metastasis right ovary	
Klotz ²⁴	30	6	1½ years	right ovary	
Voigt ²⁵	43	4	12 years	left ovary	
Fairbairn ²⁶	25	3	2 years	left ovary	
Sunde ²⁷	?	?	?	ovary	
Risel ²⁸	25	3	8 months	right ovary	
Dougal ²⁹	29	2	1 year	right ovary	
Ries ³⁰	48	1	10 years	right ovary	
Kynoch ³¹	29	multipara	8 weeks	left ovary	
Phillips ³²	25	1	2 years	right ovary	
Kleinhans ³³	?	?	?	ovary	

TABLE II
Chorionepitheliomas of the Ovary without previous Pregnancy

Author	Age in years	Localization	Remarks
Lubarsch ³⁴	13	tumor of the abdomen connected with the uterus	no necropsy
Rosner-Glinski ³⁵	18	ovary	tubal pregnancy
Pick ⁶ (first case)	30	dermoid of ovary with chorionepitheliomatous proliferation	
Pick ⁷ (second case) ..	9	teratoma and chorionepithelioma	
Varo ³⁶ (first case)	18	right ovary	
Varo (second case) ...	26	left ovary	
Kaufmann ³⁷	?	ovary	

TABLE III
Questionable Ovarian Chorionepitheliomas

Author	Age in years	Localization	Remarks
Michel ¹⁷	16	cancer of ovary chorionepitheliomatous metastases	
Schmaus ¹⁶	16	cancer of ovary resembling chorionepithelioma	
Bock ³⁸	13	chorionepithelioma originating from ovarian cyst	
Massabuau ^{39, 40} (two cases).	?	cancer with chorionepithelioma-like parts	

I. PRIMARY OVARIAN CHORIONEPITHELIOMA

Report of Case

CASE I. (*Clinical report.*) A woman aged 26; one child living; no history of abortion; date of last delivery unknown. Onset of illness marked by violent headaches and severe left facial pain four weeks before admission. Two weeks later difficulty in swallowing and regurgitation of food through the nose. On entry, weakness, difficulty in swallowing, vertigo. Patient poorly developed and emaciated; skin yellowish; mucosae anemic. Heart and lungs negative; liver 1.5 cm. below costal margin. Examination of nervous system reveals normal eye grounds; eye movements free; right nystagmus; paresis of soft palate; uvula in midline; decreased palatal, patellar and Achilles reflexes; paresis of left recurrent laryngeal nerve. Leucocytes 21,000; red blood cells 2,600,000; hemoglobin 40 per cent; color index 0.77. Death four days later. Clinical diagnosis: brain abscess.

NECROPSY REPORT

The body is that of a poorly developed, emaciated female; the skin is pale and slightly yellowish. Several raised, cyanotic nodules about 0.5 cm. in diameter are distributed over the skin. On incision the nodules are soft, circumscribed and appear quite vascular.

Heart: Negative.

Lungs: Many nodules resembling those in the skin are present in both lungs; and in the lower lobe of the right lung there is a tumor mass about 3 cm. in diameter that projects 0.5 cm. above the pleural surface. This tumor has a spongy consistence, its center is depressed, its cut surface shows grayish to cyanotic mottling and varying sized blood spaces; all in all, the nodule resembles placental tissue.

Kidneys: Both show several small cyanotic nodules.

Liver: The liver edge is somewhat above the umbilicus; the surface is roughened by spongy cyanotic nodules 0.5 to 2 cm. in diameter; in the right lobe there is a large nodule 6 cm. in diameter that closely resembles placenta.

Pancreas: A considerably smaller nodule occupies the head of the pancreas.

Bladder: No neoplastic foci are visible in bladder.

Genital Organs: The uterine cavity is 5.5 cm. long, the mucosa is smooth, and there is no evidence of tumor. The fallopian tubes are thin-walled with patent abdominal ostia. The right ovary measures 2 by 2 cm. and contains a few small cysts. The left ovary measures 6 by 3 cm.; the capsule is grayish and smooth except for a few nodules 0.5 to 2 cm. in diameter. The organ is not adherent to the surrounding structures. On section the entire ovary is composed of cyanotic, cavernous, spongy tissue resembling placenta.

Head: In the skull are three similar nodules; in the brain, which is somewhat edematous, two small cyanotic patches, penetrating the brain to a distance of 2 to 3 mm., are visible over the convexity. Cyanotic, fragile tissue extends into the left sigmoidal sinus, and just posteriorly the dura is pierced by an identical layer 2 to 3 mm. thick. Similar tissue fills the left middle ear; and the lymph nodes about the left carotid artery are likewise involved.

MICROSCOPIC REPORT

Ovaries: Left ovary is surrounded by a fibrous capsule containing many small vessels and minute hemorrhages. No tumor tissue penetrates the capsule. The greater portion of the ovary consists of necrotic tissue in which the outlines of many vessels and larger blood-filled sinuses are apparent. The vessel walls present a homogeneous structure because of a fibrinous transudate, and in the larger sinuses a fibrin network suggests a thrombus. In these areas are shadows of large cells with a faintly eosin-staining cytoplasm but without nuclei, although some chromatin debris is present. The cytoplasm of some cells contains brownish pigment. Leucocytes with fragmented nuclei infiltrate the necrotic foci. In the periphery of the tumor mass the tissue is satisfactory for study. This tissue is very vascular; many capillaries contain fibrin threads, and in a few larger vessels

thrombi are present. In places the capillary endothelium is swollen and the nuclei are vacuolated. In the meshes of this capillary network are masses of hemorrhagic tissue consisting largely of polygonal cells of different sizes, and in places closely packed together in small aggregates. The nuclei are often swollen or hyperchromatic; the cytoplasm is faintly acidophilic. Many mitotic figures are present, some abnormal in character. Interspersed amid these cells are less numerous, large, deeply staining cells containing two to three or more dark oval nuclei. Their shape is varied; many of these syncytial cells are adjacent to vessels and between the two cell types transitional forms may be seen. Penetration of vessel walls by tumor cells is not observed, but nevertheless large polygonal tumor cells, some with poorly stained nuclei, are found in vessel lumina. No ovarian tissue remains nor is there any suggestion of teratoma.

Kidneys: The centers of the metastatic nodules consist of blood-filled sinuses, fibrin threads and necrotic cells. Living tumor tissue resembling that found in the ovary forms the periphery of the nodule; but here syncytial cells predominate, whereas in the ovary the Langhans cells are more prominent; the syncytial cells are invading between renal tubules; some peripheral lymphocytic infiltration is present.

Pancreas: There is a similar histologic picture; pancreatic tissue is infiltrated by syncytial cells.

Liver: The same tumor structure is present with syncytial cells invading between the cells of the parenchyma. There is a peripheral lymphocytic infiltration with some increase in Kupffer cells but no evidence of transformation of these cells into syncytial cells (Bostroem). The surrounding capillary shows no proliferation.

Lungs: The tumor nodules present a similar appearance as vascular necrotic foci and a periphery of large polygonal mononuclear cells and syncytial cells. The latter infiltrate the surrounding compressed lung tissue. Smaller areas consist mostly of syncytial cells with a few scattered Langhans cells. In one medium-sized vessel both types of tumor cells are found in the clot.

Genital Organs: Examination of numerous portions of vagina, uterus, tubes and parametrial structures fails to reveal tumor or evidence of decidual reaction. The right ovary shows follicles, corpora albicantia and a few serous cysts with lutein cells and proliferating theca cells in their walls.

DISCUSSION

What conclusion may one draw from this case? May we call it one of primary ovarian chorionepithelioma? There are three possibilities: (1) Chorionic elements were deposited in the left ovary, liver, lungs and other organs without there having been a primary uterine tumor, and these deposits have given rise to multiple tumors; (2) there was at one time a primary uterine tumor which metastasized, whereupon the primary tumor either healed or was expelled; (3) the ovarian tumor is primary and the other nodules metastatic. In this latter event it may be variously interpreted.

It is very difficult to confirm any one of these three possibilities. The first seems most unlikely, as it is hard to imagine such numerous deposits of chorionic material all giving rise to tumors. Nor can we definitely exclude the second supposition since we know (Münzer²¹) that ovarian metastases of uterine chorionepitheliomas, though rare, do occur. In our case, with metastases so numerous, and with one ovary so completely involved, we should expect both ovaries to be invaded, but on the contrary, the other ovary contained no tumor tissue. The ovarian tumor mass was single, discrete and encapsulated and was the only genital localization of a characteristically genital neoplasm. We, therefore, consider it justifiable to regard it as the primary tumor.

As a primary ovarian tumor it may have originated (1) after pregnancy — like an ectopic chorionepithelioma; (2) as a one-sided development of a teratoma; (3) in connection with an ovarian pregnancy. The first supposition might or might not presuppose previous mole or uterine tumor expelled by labor, leaving no trace. The second hypothesis is difficult to exclude since Pick has been able to demonstrate this possibility. The teratomatous portion may have undergone necrosis; nevertheless, in testicular chorionepitheliomas, teratomatous portions, especially neuroepithelial portions, are readily demonstrable. We believe that after puberty we may consider chorionepitheliomas as being teratomatous in origin only where such parts can be demonstrated or where pregnancy can be absolutely excluded. Previous to puberty all cases should be regarded as teratogenous.

Klotz²⁴ regarded his chorionepithelioma as an outgrowth of teratoma but could not demonstrate teratoma; in addition, there

was a history of delivery eighteen months previously. It is probable that he was dealing with ectopic chorionepithelioma. Voigt²⁵ likewise did not find evidence of teratoma, but since the last pregnancy had occurred twelve years previously, he believed that could be excluded. However, there are cases reported with even longer latent periods, hence his case also was probably an ectopic tumor. It is very questionable whether the cases of Schmaus and Michel (cancer of the ovary with chorionepitheliomatous proliferation), and that of Bock³⁸ (chorionepithelioma originating from juvenile cystoma of the ovary) are real chorionepitheliomas. The same is true of the observations of Massabuau^{39,40} who likewise reported chorionepitheliomatous foci in two ovarian cancers. The latter author explained the syncytial cells according to the theory of Chevassu,⁴¹ namely that rapidly growing cancer cells penetrate blood vessels and, due to their intimate vascular connection, become transformed into syncytium-like cells. Pick found an ovarian dermoid with chorionepitheliomatous proliferation and in addition a tubal pregnancy of the same side. Pick considered the case primarily teratogenous but Risel²⁸ pointed out that penetration of a preëxisting dermoid by chorionic tissue from the tubal pregnancy could not be excluded. The third supposition (relation to ovarian pregnancy) may now be considered: Kleinhans could not exclude ovarian pregnancy in his case; Sunde regarded his as having developed from an ovarian pregnancy because he actually found villi. In our case we have neither evidence of teratoma nor ovarian pregnancy, therefore, we must regard it as one of primary ectopic chorionepithelioma following a previous pregnancy. Whether or not a mole existed at the time we cannot tell; but from the work of Marchand and Pick, which is supported by many observations, we know that ectopic chorionepitheliomas may occur even after normal pregnancy.

II. PRIMARY CHORIONEPITHELIOMA OF THE LIVER

There is very little in the literature on primary chorionepithelioma of the liver. Gurewitsch⁴² reported the first case as follows: Patient, a woman, 31 years of age, died eighteen months after a hydatidiform mole. Tumor nodules were found in the liver, one invading the small intestine. Other neoplastic foci in lungs and mesentery. No evidence of tumor in the uterus but decidual changes present; lutein cysts in the ovaries.

A second instance was reported by Fischer.⁴³ The patient, aged 45 years, had had two deliveries, the last eighteen months previous to admission. Necropsy disclosed numerous nodules in the liver varying from 2 to 6 cm. in diameter. There was tumor invasion of the hepatic veins. One similar nodule was present in the head of the pancreas. Microscopic examination revealed a typical chorionepithelioma. The uterus contained no tumor, nor was any found in the lungs. No teratomatous portions were demonstrable. The author believed that the tumor was related to the last pregnancy and that chorionic elements had become deposited in the liver and developed by a process of retrograde metastasis. The last mode of transmission is favored by Fischer in view of the frequency of retrograde spread in chorionepitheliomas of the vagina. Christeller and Oppenheimer⁴⁴ observed in a woman 52 years of age, with a history of ten normal deliveries, the last of which dated back twelve years, a liver weighing 2000 gm., filled with chorionepitheliomatous nodules, the largest 6 cm. in diameter. Smaller foci occurred in the lungs and intestinal wall. No uterine tumor was found; the uterine mucosa showed decidual changes, and lutein cysts were present in the ovaries.

Stoy⁴⁵ found chorionepitheliomatous nodules in the liver of a man of 40 years; metastases (regarding the large liver nodule as primary) were present in lungs, gastric and intestinal mucosae, spleen and perihepatic nodes. No testicular tumor could be demonstrated. The author regarded the case as an overgrowth of chorionepitheliomatous elements in a liver teratoma but did not find a teratoma histologically. Serial sections, however, were not examined.

Albrecht⁴⁶ found chorionepitheliomatous portions in a case of hepatic teratoma but nevertheless does not regard it as true chorionepithelioma. Marx⁴⁷ described one case of angioplastic liver sarcoma, that of a man 52 years of age. This case is mentioned because Bostroem¹⁸ regarded it as one of chorionepithelioma. Our examination of Marx's paper gave us no evidence for such a conclusion.

REPORT OF CASE

CASE II. (*Clinical report.*) Patient a woman, aged 46; menses began at 15 and continued regularly up to 35 at which time they ceased; five pregnancies; two children living and well. In February 1920 she observed a small tumor in the right abdominal region which about ten months later occupied the entire

right abdomen. In January 1921 the patient entered the hospital, and a large mass, presumably liver, was found extending 8 cm. below the costal margin. Aside from atrophy the genitalia were negative. Luetic scars on the lower limbs, and a strongly positive blood Wassermann led to a diagnosis of multiple liver gummas and antisyphilitic treatment was begun; the patient, however, became more cachectic and the nodular hepatic tumor increased in size. She died the following month.

NECROPSY REPORT

Peritoneal Cavity: It contains 300 cc. of clear, yellow fluid. The liver is greatly enlarged, the lower pole of the right lobe being 3 cm. above the iliac crest. The transverse colon is adherent to the inferior surface of the liver.

Pleural Cavities: The right pleural cavity contains 200 cc. of clear fluid.

Lungs: Two very small bluish brown nodules lie in the upper portion of the right lower lobe; and the lower lobe of the left lung contains several similar nodules.

Liver: The liver is enlarged to about three times its normal size, and is studded with brownish blue nodules varying from 1 to 7 cm. in diameter. The fresh surface shows that most of the tumor occupies the right lobe which is composed almost entirely of fused irregular areas of tumor tissue; only three small nodules are found in the left lobe. The nodules, which vary considerably in size, are soft, fragile and somewhat spongy. The centers of the larger nodules are yellowish, the outer portions are brownish blue, while the surrounding stroma is fibrous and depressed.

The gall bladder is enlarged and filled with thick brownish bile. A small tumor nodule lies beneath the mucosa in the region of the neck of the gall bladder. The mucosa covering the mass is elevated and soft. There are about fifty small concretions but the bile ducts are patent.

Kidneys: The surface of the left kidney shows one small focus of tumor tissue.

Genital Organs: There is a small hemorrhagic cyst in the left ovary. The vaginal and uterine mucosa is negative.

MICROSCOPIC REPORT

The central portions of the tumor nodules in the liver consist largely of blood-filled sinusoids with much fibrin, giving a thrombus-like appearance. The hemorrhagic necrotic centers are surrounded by zones of tumor cells; these cells are of two types, (1) large polygonal cells with many mitoses and vacuolated nuclei, and often showing an epithelial arrangement, and (2), darkly staining, multinucleated syncytial cells, which infiltrate between columns of liver cells. Both types infiltrate vessel walls where syncytial cells are found beneath the endothelium; the lumen of one of the larger vessels is completely filled with tumor cells. The Kupffer cells show no tendency to proliferate and form syncytial cells, which is contrary to Bostroem's hypothesis. The liver cells between the tumor areas are faintly vacuolated but their nuclei are well preserved.

Lung: The smaller nodules seen in the lung are not necrotic and consist of scattered Langhans and syncytial cells intermingled with blood sinuses.

DISCUSSION

Is this case to be regarded as primary chorionepithelioma of the liver? We believe the affirmative since no primary uterine tumor could be found and since the largest mass of tumor occurred in the liver. The small nodules in the lung and left kidney are most likely metastases of the liver tumor, it being scarcely conceivable that all the masses should be metastases of another undiscovered tumor. We cannot settle without difficulty the origin of the tumor. No teratomatous elements were observed, yet the possibility of such an origin cannot be disproved as the chorionepithelioma may have completely overgrown and masked a teratoma. The patient was pregnant five times, so there is the probability that the tumor is ectopic chorionepithelioma developing in a retrograde fashion as described by Fischer. We do not know the date of the last delivery but from the history there had been no menses for eleven years. Even though this might represent the latent period of the tumor's development, for longer latent periods are on record, it is not an absolute proof against the hypothesis of an ectopic origin.

SUMMARY

1. Two cases of ectopic chorionepithelioma are reported, one a primary chorionepithelioma of the ovary, the other of the liver.

2. In the case of the ovarian neoplasm no other genital focus existed. Metastatic nodules were found in the skin, lungs, kidneys, liver, pancreas, brain and left temporal bone. After discussing the possibilities as to the origin of ovarian chorionepitheliomas, this case is believed to fall into the class of ectopic chorionepitheliomas.

3. In the case of the liver chorionepithelioma no primary genital tumor was found and but few and small metastases were observed in the lungs and in the left kidney; the liver, however, was considerably enlarged and contained huge masses of histologically typical chorionepitheliomatous tissue. No teratomatous elements were demonstrable. The case is likewise, therefore, regarded as one of ectopic chorionepithelioma initiated by the transportation in a retrograde manner of chorionic elements through the inferior vena cava.

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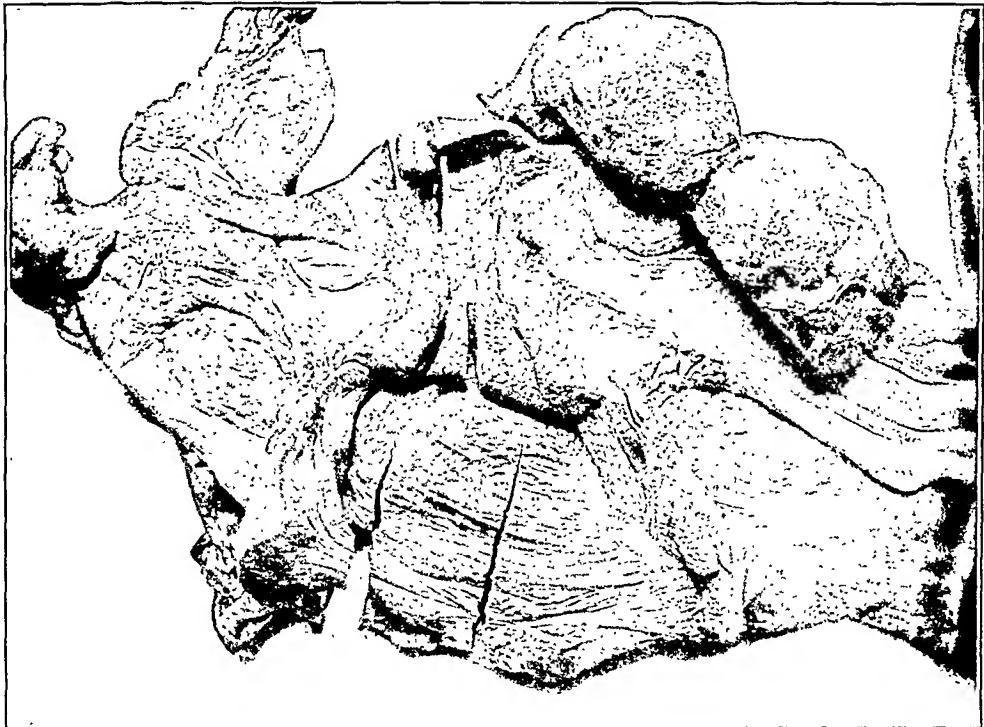
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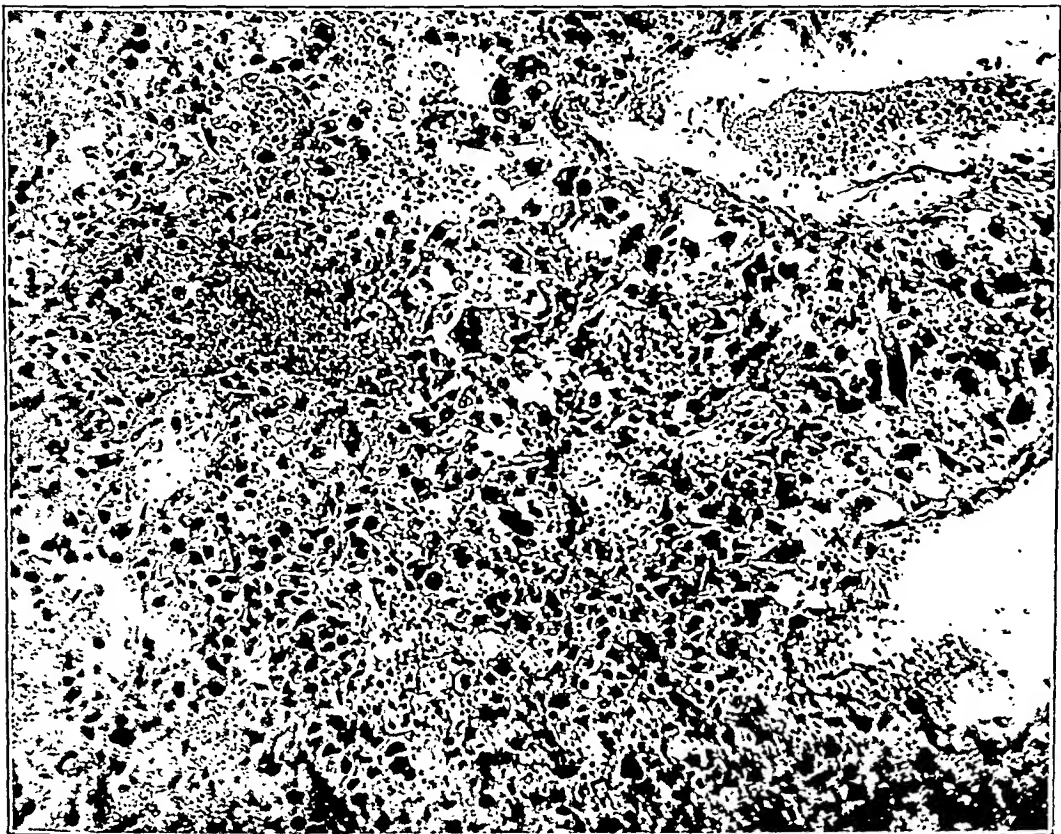
DESCRIPTION OF PLATE

PLATE 18

- FIG. 1. Case of primary ovarian chorionepithelioma. Genital organs after hardening in formalin. The left ovary consists of sponge-like tumor tissue.
- FIG. 2. Case of primary ovarian chorionepithelioma. Photomicrograph of section from periphery of the tumor of the ovary. Two different types of cells (Langhans and syncytial) are demonstrable, as well as large sinuses filled with blood.



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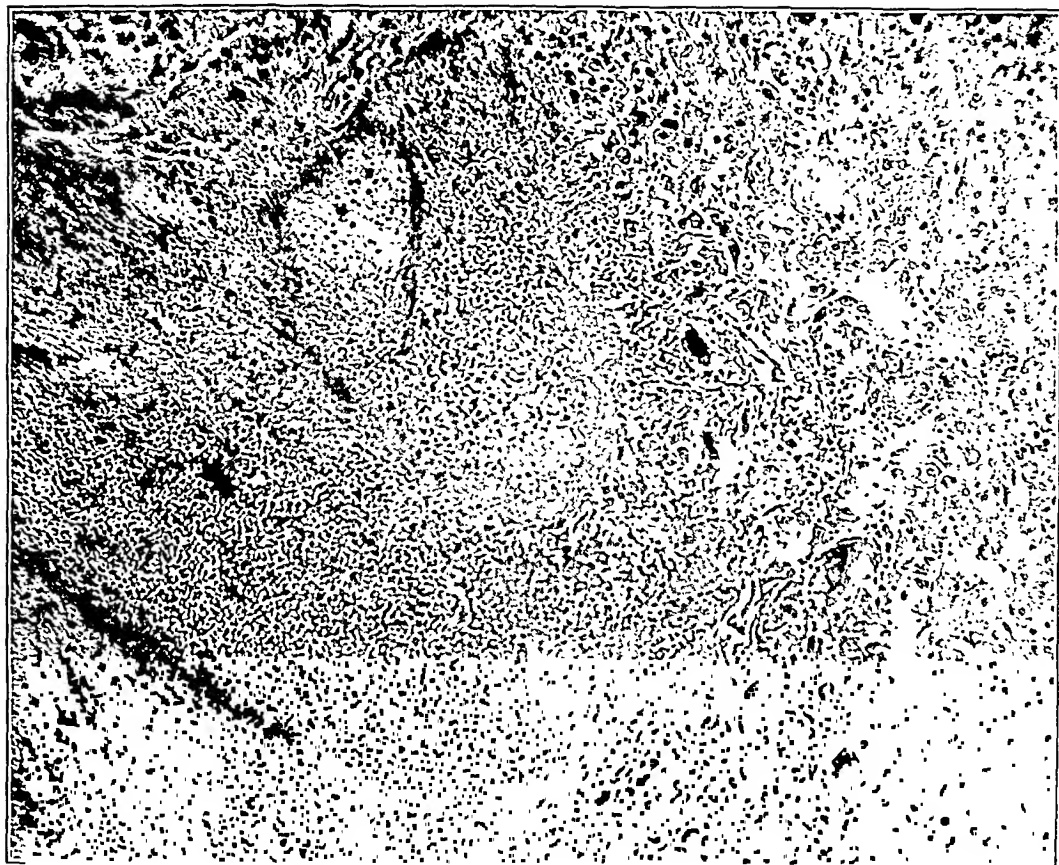


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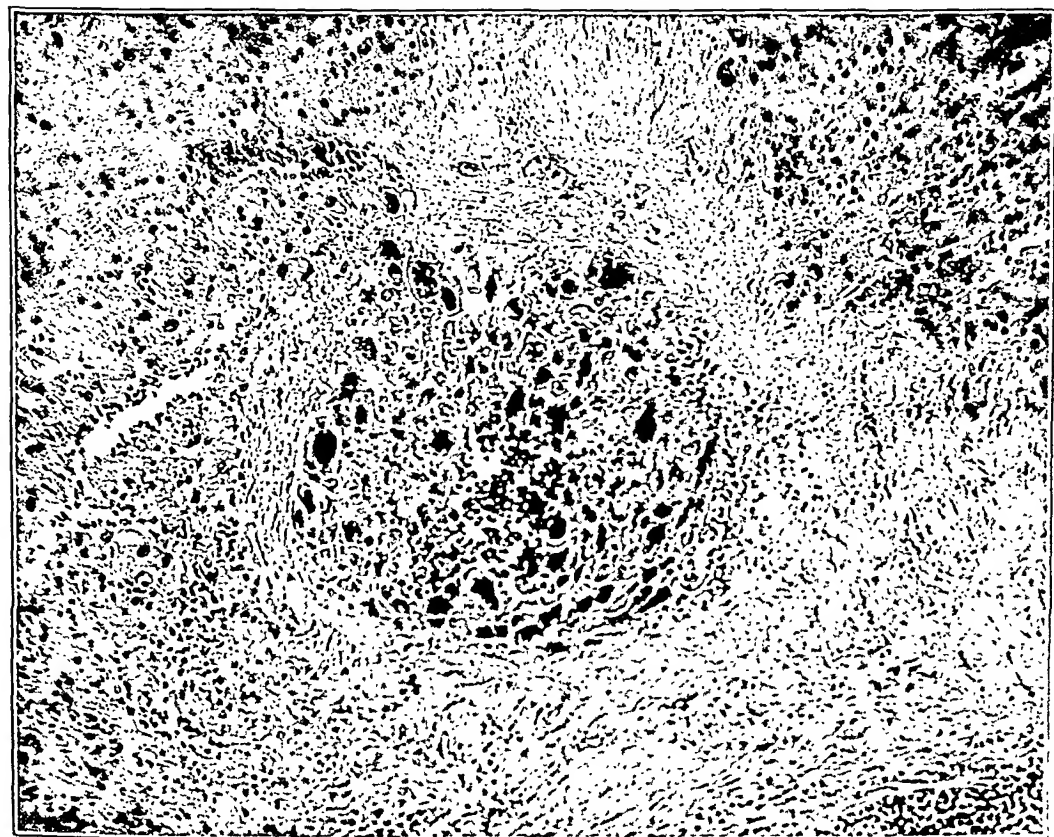
PLATE 19

FIG. 3. Case of primary hepatic chorionepithelioma. In the tumor are large sinuses filled with blood and fibrin threads. In the periphery of the tumor are large syncytial cells.

FIG. 4. Case of primary chorionepithelioma of the liver. A blood vessel of the liver filled with tumor cells.



3



4

MUSCLE HEMOGLOBIN IN HUMAN AUTOPSY MATERIAL *

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Experiments in this laboratory have shown that for the dog, muscle hemoglobin is a variable factor.¹ It is in highest concentration in adult active dogs and lowest in young pups. It is probable that exercise is more important than the level of the blood hemoglobin in determining the hemoglobin concentration in muscle fibers. Severe long-continued anemia in dogs may reduce the muscle hemoglobin level somewhat, but in healthy anemic adult dogs it rarely falls below 400 to 500 mg. muscle hemoglobin per 100 gm. We may observe lower normal values in quiet, confined, non-anemic, adult house dogs.

A method satisfactory for dogs was described in detail and we thought it would be possible to modify this muscle hemoglobin method sufficiently to give worth-while determinations in human necropsy material. In dogs it is possible to render the muscles practically blood hemoglobin-free by simultaneous bleeding and perfusion, massage and careful washing.² There are many difficulties in the necropsy procedure and these results of necessity are not so satisfactory as were the experiments in dogs. Access to muscles is limited in the usual postmortem examination and most of our readings concern the rectus abdominis and adductor longus muscles.

We have shown that in dogs it is necessary to free the muscle of contained blood hemoglobin in order to obtain accurate values, and it must be admitted that only occasionally will necropsy material be found which can be prepared free from blood hemoglobin. Moreover, it is not difficult to show that under certain conditions muscle hemoglobin can be washed out of the muscles by an excess of gravity perfusion. For example a dog perfused on one side immediately after death may be placed in the cadaver ice-box forty-eight hours. The perfusion of the other side with pressure, using the standard glucose salt solution, will give distinctly lower readings in the late

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perfused side in spite of the fact that some blood clots still persist in the smaller vessels of these muscles. This indicates obviously that muscle hemoglobin has been washed out of these muscles perfused forty-eight hours after death. It is possible that physical changes in the muscle hemoglobin and muscle fibers due to rigor mortis, accumulation of lactic acid or other factors are in part responsible for this escape of muscle hemoglobin in the perfusion fluids.

In spite of these many difficulties we decided to proceed with a study of the human material. Care must be used in the interpretation of results and no emphasis can be placed upon minor variations in the muscle hemoglobin readings, but we believe the larger variations in muscle hemoglobin values do have some significance. It is significant that these human values and abnormalities coincide with the more easily controlled observations in dogs. It appears that the level of muscle hemoglobin in man as in the dog is very largely determined by exercise or work done. Probably low blood hemoglobin values and diet factors have some little influence on the level of muscle hemoglobin.

METHOD

The results reported herein were compiled using the adductor longus and the rectus abdominis muscles. A few minor modifications were made in the method during the study, but essentially the method employed at all times was to wash through the right external iliac artery 4000 cc. to 7000 cc. of 1 per cent sodium chloride plus 5 per cent glucose solution. This washed out the blood in the vessels in the right leg and the right rectus abdominis. The muscles were not massaged either *in situ* or after removal as it invariably caused an increase in the amount of edema. It was also found that the washing if done slowly with a moderate amount of pressure was more satisfactory than if done quickly with high pressure. A four-liter bottle was used and the pressure supplied by a hand pump. It was not unusual to take from twenty minutes to one half-hour for the actual washing. Of the utmost importance was the length of time which had elapsed since death. In those cases in which the blood had clotted in the vessels it was impossible to remove any great amount of blood, and even small amounts of perfusing fluid

gave edema, while larger amounts frequently seemed to wash out a certain amount of muscle hemoglobin.

The right side having been perfused, approximately 30 gm. samples were removed from both adductor longus and rectus abdominis muscles, the left being kept as controls. In taking these sections due care was exercised to avoid contamination of the muscle with blood, all vessels being ligated before cutting. At first, samples were taken from each of the four muscles for microscopic examinations as to the blood content. Later sections were taken only from the muscles which had been perfused. Microscopic sections have shown that in nearly every case there is much less blood in the capillaries of the perfused side than in the unperfused side, and in some cases the perfused capillaries are entirely clear of red blood cells.

The remaining portions of the muscle were trimmed free from fat, fascia and any large blood vessels and were then chopped moderately fine with a razor blade, using this method to cut the fibers without crushing them. Duplicate 10 gm. samples from each muscle were taken and 40 cc. of 0.4 per cent ammonium hydrate added to each. The remainder of the chopped muscle was set aside temporarily. The ammonia extract was thoroughly stirred and placed in the ice-box at 4° to 7° C for sixteen hours. During this time the extract was thoroughly stirred two to three times. It was now either filtered or centrifugalized. If filtered it was first put through a wire gauze strainer, then several thicknesses of cheesecloth and then through double thickness of filter paper giving a clear filtrate. Centrifugalization at high speed for twenty minutes and removal of the supernatant fluid by pipette gave equally clear solutions and was especially advantageous in those muscles particularly rich in fat. In cases in which the centrifugalized fluid was not absolutely clear it was put through filter paper. The filtrate was a 20 per cent extract of the muscle, and because of the dilution was unsuitable for analysis by the oxygen capacity method of Van Slyke, the percentage of error due to the physically dissolved oxygen being too high.

Three different quantitative colorimetric estimations of pigment were utilized. The clear extract was examined in the spectrophotometer and the concentration estimated from the density. Kennedy,³ and Kennedy and Whipple⁴ have discussed the relation between the concentration of hemoglobin and its optical density and the identity

of blood hemoglobin and muscle hemoglobin. Small portions of the extract were taken and the oxyhemoglobin changed to carbon monoxide hemoglobin by bubbling illuminating gas through the solution. This was then read in a Duboscq colorimeter against a 1 per cent solution of dog's blood, the hemoglobin content of which had been determined by the Van Slyke method. There was frequently some difficulty in matching the solutions, the muscle hemoglobin seeming to have a slightly more yellow tint than the blood standard. To obviate this difficulty a Wratten light filter No. 74 was

TABLE I

Comparison of Hemoglobin Values on Identical Muscle Samples Estimated by Different Methods. Muscle Hemoglobin Expressed in Mg. per 100 Gm. Muscle.

	Acid hematin	CO hemoglobin		Spectrophotometer	Average
		No filter	With filter		
Left adductor not perfused.	1190	1425	1035	1270	1220
	1190	1400	1010
Right adductor perfused.	870	810	730	835	815
	880	790	790
Left rectus not perfused.	810	1000	710	780	825
Right rectus perfused.	600	710	530	570	600
	620	680	470
Right pectoral not perfused.	1130	1290	1025	1110	1160
	1210	1380	1000

placed in the eye piece of the colorimeter. Under these conditions the colors of the solutions were more nearly comparable. Kennedy⁵ has discussed the value of these light filters in colorimetry. The remaining portions of the original muscle extract were now changed into acid hematin and read against a similar standard prepared from dog's blood. The solution is prepared as follows: Add 4 drops of 5N hydrochloric acid to 10 cc. of the extract, shake until the precipitate is redissolved, and then place in the ice-box over night.

The pigment content of the muscle of ten cases was determined by using three colorimetric methods; namely the *spectrophotometric*, the *acid hematin*, and the *carbon monoxide hemoglobin* both with and without the aid of light filters. Table I shows the relative agreement of these four methods on one case (A-209). In our hands the determination by the carbon monoxide method was the most troublesome

and it was thereafter dispensed with. All other figures appearing in this paper are averages of determinations by the acid hematin and the spectrophotometric methods, each of these usually made by a separate observer * and all of them averages of duplicate examinations.

Since even with the greatest care edema occurred in certain cases it was thought wise to make a correction for this in so far as was possible. From the original chopped muscle, duplicate 1 gm. samples were weighed into weighing bottles and dried to constant weight in a vacuum oven with a temperature of 72° to 76° C and a vacuum of from 23 to 27 inches of mercury. As a rule, at the end of ten hours the specimens were so dehydrated that another six to ten hours drying did not change the weight more than 1 to 2 mg. Correction for edema may be readily calculated from these figures. As there was no assurance that the rectus and the adductor muscles were equally perfused and equally edematous, it was necessary to determine each of them separately on each side and each in duplicate. This method is faulty in many respects because the perfusing fluid itself contained 6 per cent of solids, and there is no assurance that during the perfusion a certain amount of protein might not have been washed out of the muscles. So in addition to estimating the relative dry weights of the different samples the amount of nitrogen was determined on the dried samples by the Kjeldahl method. On this basis the protein content was estimated and a correction established for the edema. In practice, for any pair of muscles, we usually averaged the factor obtained by comparing the dry weights and the factor obtained by comparing the protein content, and used this average factor to correct for edema of the muscles. Table II (page 80) illustrates the points mentioned.

Usually the correction factor is greater than 1. It is always greater than 1 where there is edema due to perfusion. However, in a certain percentage of our cases the correction factor is less than 1. For example, in a case such as A-296 where death was due to myocardial failure with marked anasarca, it is probable that the hypertonic perfusing solution caused absorption of fluid from the tissues.

It may be recalled that the spectrophotometric curve of the absorption bands of muscle hemoglobin is just slightly but definitely

* We wish to acknowledge the assistance of Miss Beatrice Moshier, who made most of the acid hematin readings.

different from that of blood hemoglobin, as pointed out by Kühne,⁶ Günther⁷ and others. The difference is that the points of maximum absorption of each of the two main bands in the muscle hemoglobin are moved about 5 microns toward the longer wave end of

TABLE II

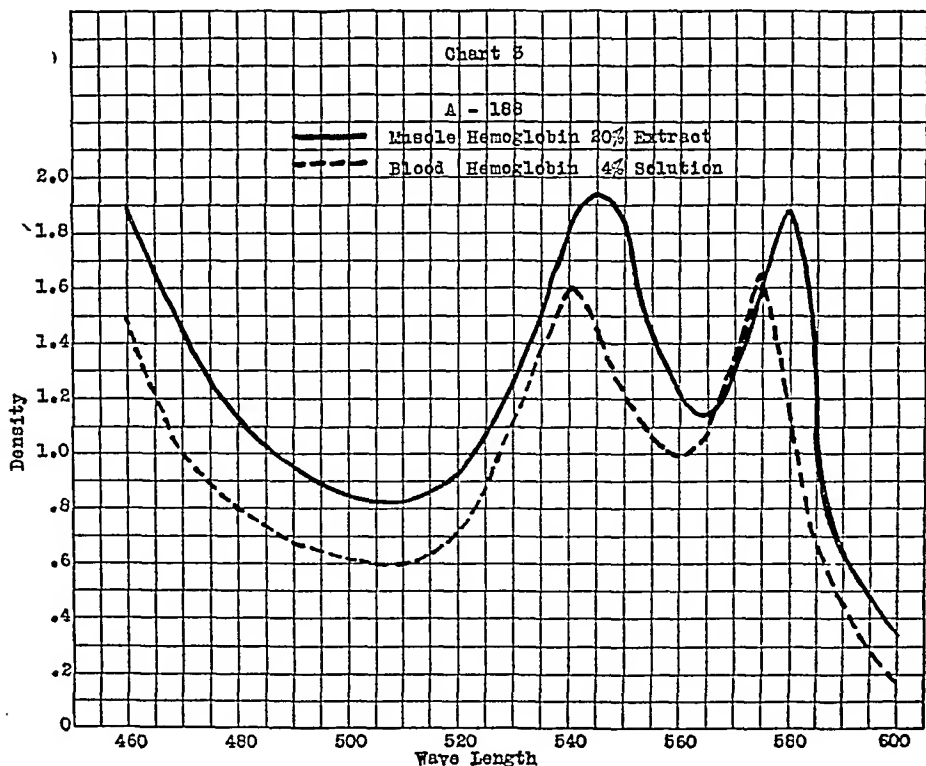
Method of Calculating Correction for Edema in Perfused Muscle (A-258)

1. COMPARISON OF DRY WEIGHTS						
		Wt. of muscle in gm.	Dry wt. in gm.	Per cent dry weight	Average	
Right rectus perfused	A ...	0.824	0.172	20.9	20.8 $\frac{25.0}{20.8}$ 1.2	
	B ...	0.976	0.202	20.7		
Left rectus not perfused	A ...	0.702	0.174	24.8		
	B ...	0.641	0.161	25.1		
2. COMPARISON OF PROTEIN CONTENTS						
		Wt. of muscle	Protein in gm.	Per cent of protein	Average	
Right rectus perfused	A ...	0.824	0.139	16.9	16.6	
	B ...	0.976	0.160	16.3		
Left rectus not perfused	A ...	0.702	0.155	22.1	$\frac{21.7}{16.6}$ 1.31	
	B ...	0.641	0.137	21.3		
3. CALCULATION OF HEMOGLOBIN CONTENT IN MG. PER 100 GM. OF MUSCLE						
	Duplicate samples	Right rectus perfused	Average			
Spectrophotometer.....	A	770	765			
	B	760				
Acid hematin.....	A	730	$\frac{725}{745} \times 1.25 = 930$ mg. of hemoglobin per 100 gm. muscle corrected for edema.			
	B	720				

the spectrum. This fact gives us an opportunity to test in a gross way the importance of the blood hemoglobin in the capillaries in any given muscle sample. Chart 3 presents the curves of the blood

hemoglobin and muscle hemoglobin from the same case (A-188). These curves are presented as they are entirely typical of the hemoglobin from the two sources. In this particular case the blood hemoglobin was estimated one month before death. The two readings were made by separate observers. The muscle hemoglobin was estimated from an unperfused muscle which still contained blood in the

CHART 3



The Curves of the Blood Hemoglobin and Muscle Hemoglobin (A-188)

capillaries. It will be seen, however, that this particular case was one of very severe anemia and the hemoglobin content of the blood was much lower than normal. However, we have repeatedly observed this same type of muscle hemoglobin curve in both the perfused and unperfused muscle.

That there is a noticeable difference between the perfused and unperfused side is shown by Table IV which gives the values of the perfused side for the adductor longus as illustrated above. Except in three instances the corrected value for the perfused muscle is lower than that of the unperfused side. The average of these is

around 10 to 15 per cent below the value of the unperfused muscle. It must be admitted that perfusion rarely removes all of the contained red cells so that this correction is an inaccurate variable. In three instances the corrected value of the perfused muscle is greater than that of the unperfused side. This point is discussed above and is probably largely due to the hypertonic perfusate in the presence of tissue edema of disease.

TABLE IV

Muscle Hemoglobin before and after Perfusion. Mg. per 100 gm. of Muscle

No.	Leg unperfused	Leg perfused	Leg perfused; corrected for edema	No.	Leg unperfused	Leg perfused	Leg perfused; corrected for edema
X-670.....	1010	805	A-268.....	975	860	840
A-172.....	1000	630	A-274.....	1050	900	900
A-188.....	1140	940	1000	A-280.....	1225	670	840
A-198.....	960	930	970	A-281.....	600	650	590
A-204.....	820	615	690	A-282.....	540	580	580
A-209.....	1230	860	1120	A-283.....	970	990	1030
A-253.....	750	790	X-835.....	570	550	550
A-254.....	1095	945	1040	A-285.....	1170	1100	1050
A-258.....	1160	910	1090	A-295.....	800	510	550
A-260.....	1015	855	A-296.....	670	660	590
A-262.....	1180	740	930	A-297.....	1050	1000	990
A-267.....	795	370	550				

Necropsy Material. In Table V we present muscle hemoglobin values together with the cause of death, age of patient and blood hemoglobin. The values for the blood hemoglobin and all other data pertaining to the patient we have obtained from the clinical history. The blood hemoglobins are recorded on the basis of the Sahli standards. It will be noted that the values range all the way from 550 mg. to 1120 mg. of hemoglobin per 100 gm. of muscle. In comparing the relationship between blood hemoglobin and muscle hemoglobin it is interesting to note that seven of sixteen cases have blood hemoglobin of 75 per cent or below. Of these seven only three have muscle values in the lower half of the group and the remaining four have muscle values in the high upper part of the group. In fact the highest muscle value obtained had a blood hemoglobin of 70 per cent while the two lowest blood hemoglobins, 30 per cent and 43

per cent, have very high muscle hemoglobin values, 1090 and 1000 mg. respectively. These two cases are quite remarkable; the lower value of 30 per cent hemoglobin was found in a case of rapidly

TABLE V

Muscle Hemoglobin as Related to Blood Hemoglobin. Cause of Death, and Age of Patient

Case No.	Muscle hemoglobin *		Age	Blood hemoglobin per cent	Red cell count	Cause of death
	Ad-ductor	Rectus				
A-267 ...	550	490	72	80	4,250	Combined sclerosis. Bronchopneumonia.
A-295 ...	550	475	58	60	2,400	Colitis. Starvation.
A-282 ...	580	550	58	77	4,910	Carcinoma of breast.
A-296 ...	590	...	53	90	Myocardial failure.
A-281 ...	590	...	77	84	Arteriosclerosis. Chronic nephritis.
A-204 ...	690	...	56	65	Perinephritic abscess. Starvation.
A-253 ...	790	710	57	90	Arteriosclerosis. Lobar pneumonia.
A-268 ...	840	...	41	70	2,800	Alcoholism. Pneumonia.
A-274 ...	900	670	55	84	Carcinoma of trachea.
A-262 ...	930	950	13	92	5,570	Scarlet fever. Bronchopneumonia.
A-198 ...	970	870	10	75	4,568	Pneumococcus meningitis.
A-188 ...	1000	1030	4.5	43	2,600	Aplastic anemia.
A-283 ...	1030	750	15	78	4,800	Post diphtheritic paralysis and bronchopneumonia.
A-254 ...	1040	...	32	95	4,650	Lobar pneumonia.
A-258 ...	1090	970	25	30	1,740	Aplastic anemia.
A-260 ...	1100	820	38	70	3,810	Post scarlatinal nephritis.

Additional cases in which blood values are not available

X-835 ..	550	...	60	Cirrhosis.
A-205 ...	620	...	43	Cirrhosis. Lobar pneumonia.
X-670 ..	805	625	45	Fractured skull.
A-280 ...	840	...	51	Strangulated hernia. Peritonitis.
A-297 ...	990	...	66	Carbuncle. Septicemia.
A-285 ...	1050	790	27	Syphilitic meningitis. Basilar hemorrhage.
A-209 ...	1120	770	40	Diffuse bronchopneumonia.

* Hemoglobin in mg. per 100 gm. of muscle. Values of perfused muscle corrected for edema.

developing aplastic anemia (A-258). The entire duration of the illness was three to four weeks in an active young woman, 25 years of age. The other case in this group is an unusual one of a young child

4½ years old who had been confined to his bed during most of a severe months illness. We should not expect such a high reading here for two reasons, first, age, and second, the long stay in bed. However, there is an interesting factor present; this patient had twenty-one transfusions of 50 to 150 cc. of blood within five weeks. There was much blood pigment in the endothelial phagocytes of the lymph nodes and spleen. It is probable that this greatly increased blood destruction and storage of pigment have been in some part responsible for this increase in muscle hemoglobin.

We hope to measure the muscle hemoglobin in cases of pernicious anemia as it is generally observed that the skeletal muscles are often very deep red in these cases. It is interesting to note that the case with the lowest muscle hemoglobin had a blood hemoglobin of 80 per cent. There are several cases with low muscle hemoglobin values which also have moderately low blood hemoglobin, 70 to 85 per cent, but these are usually in inactive individuals. Although this series of sixteen cases in which the blood findings are known is entirely too small to allow us to draw general conclusions, nevertheless the figures agree with those recorded in dogs and indicate that severe anemia causes only a slowly progressing slight decrease in the muscle hemoglobin.

Table VI is a redistribution of Table V showing the muscle hemoglobin values both in the acute and chronic diseases. In the thirteen necropsies in which we have listed an acute illness as the cause of death the average muscle value is 980 mg. of hemoglobin per 100 gm. of muscle. The lower values in this group are from, (1) an obese alcoholic, 45 years old, no occupation, who sustained a fractured skull; (2) an obese woman, 51 years old, who was also a diabetic and who died following an operation for a strangulated hernia; and (3) an emaciated woman of 41 "who looks much older," who was a drug addict and had suffered from pain and stiffness in the joints for several years. With the exception of these three patients, this group was fairly active, most of them young or middle-aged. In this group we have included a patient having carcinoma of the trachea; who died from obstruction and who had been fairly active until shortly before death.

Of the ten chronic diseases listed, the average hemoglobin value is 651. Not including the aplastic anemia of four weeks duration noted above it would be 620. The three lowest values listed were,

(1) a case of combined sclerosis who had been bedridden for seven months; (2) an obese habitual drunkard of 66 who had been doing no active work; and (3) an extremely emaciated woman of 54 who died of colitis and starvation. The difference of the muscle hemoglobin values in the acute and chronic diseases is quite striking.

TABLE VI

Muscle Hemoglobin as Related to Cause of Death

	Adductor longus Muscle Hemoglobin *	Average
Acute Diseases		
Pneumococcus meningitis.....	970	
Lobar pneumonia.....	1040	
Aplastic anemia.....	1090	
Post scarlatinal nephritis.....	1100	
Scarlet fever. Bronchopneumonia.....	930	
Alcoholism. Pneumonia.....	840	
Carcinoma of trachea and obstruction.....	900	
Post diphtheritic paralysis. Bronchopneumonia.....	1030	
Diffuse bronchopneumonia.....	1120	
Strangulated hernia, peritonitis.....	840	
Syphilitic meningitis.....	1050	
Carbuncle and septicemia.....	990	
Fractured skull.....	805	
	<hr/>	980
Chronic Diseases		
Aplastic anemia.....	1000	
Perinephritic abscess with starvation.....	690	
Combined sclerosis. Bronchopneumonia.....	550	
Arteriosclerosis. Chronic nephritis.....	590	
Carcinoma of breast.....	580	
Colitis. Starvation.....	550	
Myocardial failure.....	590	
Arteriosclerosis. Lobar pneumonia.....	790	
Cirrhosis. Lobar pneumonia.....	620	
Cirrhosis.....	550	
	<hr/>	651

* Muscle hemoglobin values expressed in mg. per 100 gm. of muscle.

Every case with an extremely low value is one in which there is a chronic wasting disease necessitating inactivity.

Muscle Hemoglobin Related to Age. There are four cases in this series below 20 years of age (see Table V). They show high values in the neighborhood of 1000 mg. There are only three cases over 60 years of age. Two of these show low values but both had chronic diseases. The highest values occur in a 40 year-old laborer who had

been working hard until six days before death, and in a 38 year-old well developed man. Other high values occur in patients whose ages are 25, 27, and 32. We believe that here again we observe the result of activity rather than of age itself, for the previously healthy young adults show the highest values.

In conclusion it may be pointed out that the behavior of muscle hemoglobin is very like that of blood hemoglobin particularly as regards the end products formed within the body. It is known ⁸ that muscle hemoglobin introduced intravenously, intraperitoneally or intramuscularly is promptly excreted in part as bile pigment in the urine. Therefore it will be of much interest to study clinical cases of myositis or other related diseases to obtain evidence as to the quantitative contribution of bile pigment arising in the striated muscles.

SUMMARY

A method is described for the quantitative analysis of muscle hemoglobin in autopsy material.

In twenty-three cases studied, values of 550 mg. to 1120 mg. per 100 gm. of muscle are found.

The muscle hemoglobin is apparently only slightly influenced by fluctuations in the blood hemoglobin.

The highest values are found in active young adults who have died after a short illness.

Low values are found in the chronic diseases and wasting illnesses. In every case in which there is an exceptionally low value there has been prolonged inactivity.

We conclude that in man as in the dog the concentration of the muscle hemoglobin within the muscle fibers is determined more by muscular activity than by the level of blood hemoglobin.

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AN EPITHELIAL CYST OF THE HYPOPHYSIS *

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This case is of interest because it is one of a small group of cystic neoplasms found in the region of the pituitary and because of the presence of an anomalous formation of small cystic cavities in the occipital lobe of the brain.

A comprehensive survey of the literature of such tumors is given by Globus¹ in his report of a teratoid cyst of the hypophysis and by Critchley and Ironside⁶ in a discussion of pituitary adamantinomas.

The tumor presented here has the characteristics of the group of neoplasms of the pituitary region considered by Erdheim and others to arise from remnants of the hypophyseal duct. In the embryologic development of the hypophysis two sources of origin are found,² one from the primitive mouth, the other from the third ventricle of the brain. The cerebral element scoops out the buccal portion to form a cup; the buccal connection disappears, but the cerebral persists as the infundibular stalk. The presence of two groups of squamous epithelial cells has often been noted on the anterior aspect of the infundibulum and on the anterior lobe of the pituitary. These are believed by Erdheim and others to be remnants of the hypophyseal duct, and the origin of certain cysts of the hypophysis.⁶ These tumors are lined with squamous epithelium, and may show variations in the type of growth, such as the formation of a "basal cell" type of carcinoma or of an adamantinoma.

REPORT OF CASE

Clinical History: The patient was a male English-American hatmaker of 48 years, who had been subject to sick headaches all his life. He had gonorrhea in 1897. In 1922 he first noticed dizziness. The present illness began in September, 1926, when he had a headache for three weeks; at the end of that time he had nausea and vomiting and was in bed for one week with what the local physician called "grippe." After this he was often drowsy and if he leaned over,

* Received for publication November 18, 1927.

became pale. About October 17 excessive urination, accompanied by great thirst, were first noticed. Both of these symptoms became progressively worse. There was also a loss of sexual desire. On February 23, 1927, he became emotionally unstable and after this the headache, nausea and vomiting became aggravated. On February 24 he was sent to a general hospital, where he remained six days, and was then transferred to the Boston Psychopathic Hospital.

Summary of Physical Examination: A rather emaciated man, with protruding underlip, who was so restless and uncoöperative that examination was difficult. Hair distribution and skin were normal. Circulatory and respiratory systems were negative. The pupils were unequal but reacted slightly to light and accommodation. Ophthalmoscopic examination showed that the disc margins were not clear, vessels were normal, no hemorrhages were seen. Visual fields roughly normal. (The patient's coöperation was poor.) The epigastric reflex was less active on the right than the left side and the right abdominal was absent. The knee jerks were equal and active. Sordes on lips. A slight temperature increase was present from admission onward, though no cause for this could be found. A provisional diagnosis of left-sided cerebral tumor, probably abscess, was made. X-ray plates of the sella turcica were negative on two examinations and though the symptoms suggested pituitary disease, physicians were loath to make a diagnosis of pituitary tumor with two negative X-ray reports.

After six days in the hospital he became excited, refused to eat and was tube fed until the time of his death, nine days after admission. March 11, 1927, he died, one and a half hours after a tube feeding. The total duration of symptoms was about six months.

NECROPSY REPORT

The skull, dura and convex surface of the brain were negative. The pituitary was soft, fluctuant, about three times its normal size and bulged upward out of the sella turcica. On removing the hypophysis from the brain, soft gelatinous yellow material oozed out of the infundibulum. It was difficult to define grossly the exact location of the cyst in relation to the lobes of the pituitary. No distortion of the structures at the base of the brain was present. The opening of the sella turcica did not appear enlarged, the floor, however, was brownish red, roughened and eroded.

Other findings were marked edema and congestion of lungs, passive congestion of spleen, and a chronic inflammatory nodule of the right testis with old adhesions to the tunica vaginalis.

MICROSCOPIC REPORT

The cyst was fixed *in toto* in formalin and serial sections were made which were stained with hematoxylin and eosin, Scharlach R and Van Gieson's stains. It was lined with stratified squamous epithelium with long papillary projections. On some portions of

the surfaces of the papillary projections rather low ciliated columnar epithelium was seen, and in other similar locations there were epithelial cells containing globules like those found in the epithelium of secreting glands. The largest papillary projection and what appeared to be the most actively growing portion of the cyst wall was situated between the anterior and posterior lobes, both of which were compressed by the cyst. Posterior lobe tissue was found in only a small number of the sections. At this point there were numerous gland-like structures resembling the pars intermedia of the pituitary and containing pinkish material staining like colloid. A few of these were seen at other places in the cyst wall. At one point there was a large cholesterol crystal included in a foreign body giant cell. In the cavity were masses of desquamated and disintegrating epithelial cells and polymorphonuclear leucocytes. No sebaceous glands or hair follicles were seen in any part of the cyst wall.

On microscopic examination of the brain another developmental defect was found: In the left occipital lobe several microscopic cysts were seen in the white matter. They were posterior to the lateral ventricle, and no connection with it could be found. The lining of the cysts was made up of cells resembling ependyma. There was considerable variation in the shape and size of the cavities. About the cysts were groups of cells similar to those of the lining but with no opening in the center. There were also dilated thin-walled blood vessels in the same region, but nothing that resembled choroid plexus.

SUMMARY

The cystic tumor described was situated between the anterior and posterior lobes of the pituitary, was unilocular and filled with soft yellow gelatinous material. It was lined by stratified squamous epithelium which formed papillary projections and which showed a tendency to variation by the presence of ciliated cells and cells containing secretion. No hair follicles or epithelial pearls were found, and no areas of calcification, or structures derived from any type of tissue other than epithelium were present.

The histologic appearance places the tumor in the group which Ewing³ designates as traceable to remnants of the hypophyseal duct. It resembles more the simple cysts of Duffy's Group I,⁵ than the adamantinomas or carcinomas.

The character of the symptoms, such as disturbance of heat regulation, polyuria and thirst may have been due to pressure on the base of the brain in the para-infundibular region. Bailey and Bremer⁴ in experimental diabetes insipidus concluded that lesions of the para-infundibular region of the hypothalamus may produce polyuria, thirst, cachexia and even rapid death as well as disturbance of heat regulation, and changes in the pulse and respiratory rate.

Another defect, quite probably a developmental anomaly, was present in the white matter of the left occipital lobe of the brain.

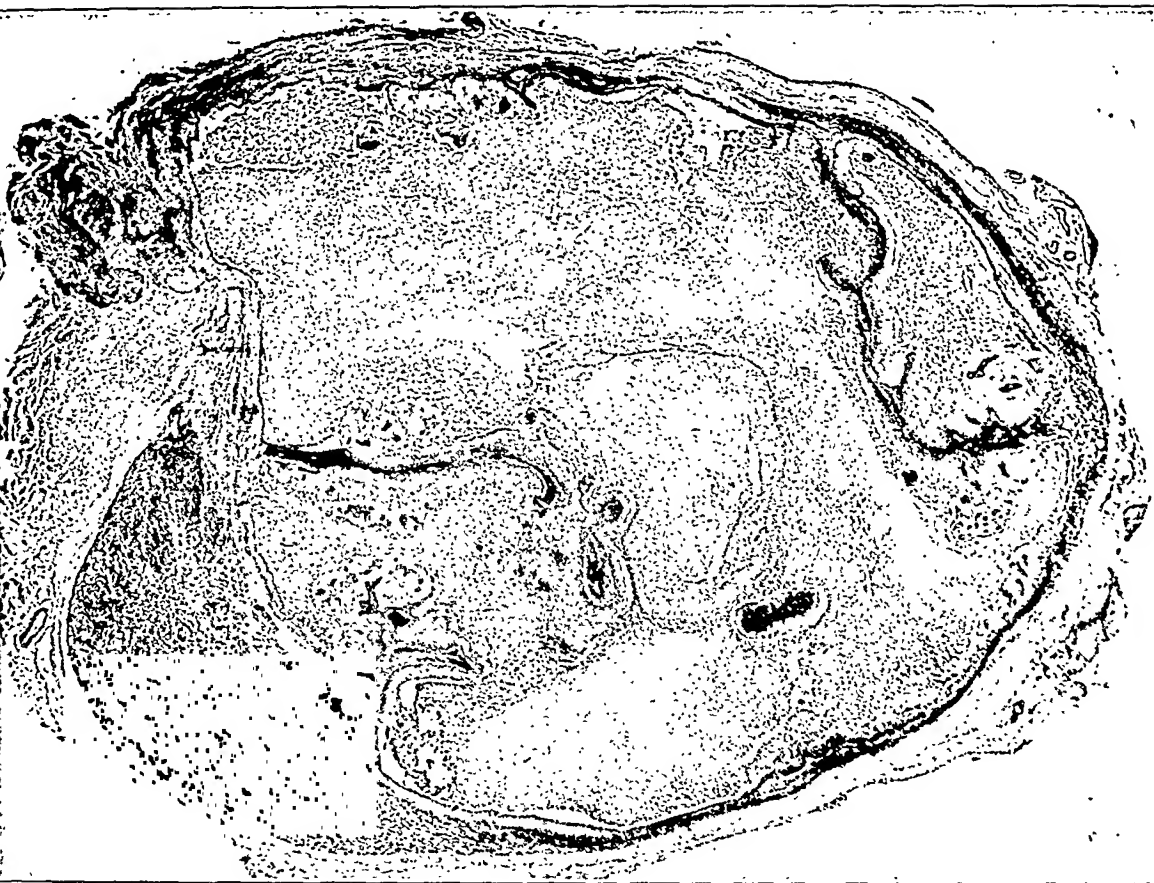
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DESCRIPTION OF PLATES

PLATE 20

- FIG. 1. Shows the relation of the dermoid cyst to the pituitary structures. Papillary projections covered with squamous epithelium extend into the cavity which is filled with desquamated epithelial cells. $\times 9$.
- FIG. 2. From above downwards the layers are as follows: necrotic, desquamated epithelial cells, stratified squamous epithelial cells lining cyst wall; connective tissue backing of cyst with formation of small papillae in places; compressed anterior lobe tissue of pituitary. $\times 75$.



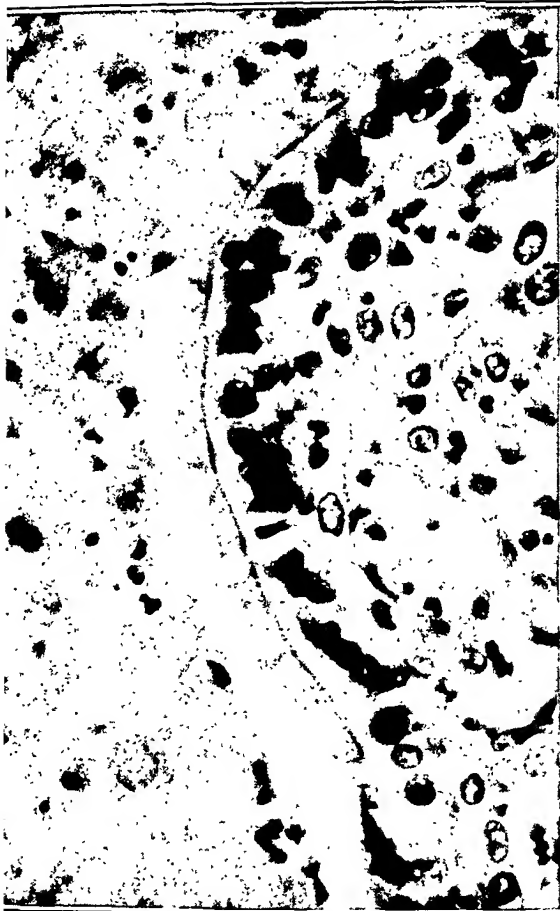
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PLATE 21

- FIG. 3. Ciliated columnar epithelium from the outer edge of a papillary projection. At one side may be seen necrotic desquamated cells and a few polymorphonuclear leucocytes and at the other, stratified squamous epithelium, covered with a layer of ciliated cells and infiltrated with polymorphonuclear leucocytes. $\times 500$.
- FIG. 4. Another variation in the type of epithelium lining the cyst; cells containing globules of mucoid secretion. $\times 500$.
- FIG. 5. Cystic cavities in the white matter of the occipital lobe of the brain. The cells lining them resemble ependyma. About the large cyst are small clumps of cells similar to those lining the cyst but without lumina. Numerous thin-walled, dilated blood vessels may be seen. $\times 45$.



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SOME GENERAL ASPECTS OF PATHOLOGICAL CONDITIONS CAUSED BY FILTERABLE VIRUSES *

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In a previous paper an attempt was made to summarize and to correlate in a general way knowledge concerning filterable viruses. At that time, facts and hypotheses regarding the nature and characteristics of the agents themselves were primarily considered. During the discussion, however, it was shown that in the majority of virus diseases a close relationship exists between the etiological agent and cells of the host. In view of this intimate type of parasitism, it seems desirable at the present time to examine carefully and, if possible, to correlate information regarding the reaction of host cells to viruses. Moreover, a knowledge of the pathological conditions produced by viruses is essential to the study of this group of etiological agents, because their existence cannot be determined, nor can their identification be established in any manner other than by the evidences of their activity exhibited in some host.

In spite of the fact that many viruses appear incapable of multiplying in the absence of suitable living host cells, it is not definitely known whether their reproduction occurs intra- or extracellularly. Nevertheless, these agents have a profound influence upon cells and produce within them remarkable changes. This influence most likely accounts for the fact that in lesions produced by many viruses the intracellular changes are sufficiently characteristic to be spoken of as inclusion bodies. In this respect a number of virus diseases differ from those caused by ordinary bacteria.

* Gross Lecture given before the Philadelphia Pathological Society, November 10, 1927.

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The inclusions have attracted the attention of many workers whose ideas concerning their nature have led to numerous discussions. It is undoubtedly true that these bodies are interesting and play an important rôle in experimental and diagnostic work, yet there are other pathological phenomena that are just as interesting as, and perhaps more important than the inclusions themselves, inasmuch as a better knowledge of them may lead to a clearer understanding of the nature of the action of viruses on cells and consequently to an explanation of why certain diseases exhibit significant inclusions while others do not. In view of the fact, however, that inclusion bodies are thought of immediately when one mentions the pathology of virus diseases, a summary of the knowledge concerning these structures will be given first and then some of the other interesting features of the pathological conditions induced by viruses will be discussed.

INCLUSION BODIES

Inclusion bodies have been seen in cells of plants, insects, fish, birds, and mammals affected by virus diseases. In some of the diseases the bodies are intranuclear, *e. g.*, in varicella and in polyhedral diseases of caterpillars; in others they are found in the cytoplasm, *e. g.*, in vaccinia and in mosaic diseases of some plants; in still others they occur both in the nucleus and in the cytoplasm, *e. g.*, in smallpox and in paravaccinia. Many of the inclusions described, however, cannot be accepted as specific or characteristic and it is these that detract from the significance of the ones well established and accepted by numerous critical observers. In Table I are listed the majority of diseases in which inclusions of one kind or another have been described. The diseases are grouped according to the location of the inclusions in affected cells.

In spite of the chaos suggested by Table I, no worker familiar with the microscopic pathology of virus diseases doubts the importance and significance of Guarnieri bodies in vaccinia, Negri bodies in rabies, Bollinger bodies in fowl-pox, polyhedral bodies in certain diseases of insects, and the nuclear inclusions seen in varicella, herpes, and several other virus diseases. Since some of the diseases in the table exhibit inclusions of significance while others do not, I have made a selection of the pathological conditions in which the inclusions appear sufficiently characteristic to be of importance. The

TABLE I

*A List of the Majority of Diseases in which Intracellular Inclusions have been Described.
The Diseases are Grouped According to the Location of the Described
Inclusions within Affected Cells.*

A. CYTOPLASM	{	Mosaic disease of certain plants
		Sheep-pox
		Contagious epithelioma (fowl-pox)
		Mollusum contagiosum
		Lymphocystic disease of fish
		Rabies
		Distemper of dogs
		Fowl plague
		Lethargic encephalitis
		Trachoma and inclusion blenorrea
		Hog cholera
		South African horse sickness
		Rickettsia diseases
		Measles
		Scarlet fever
		Cancer (malignant growths)
		Kurloff bodies (guinea pigs)
		Todd bodies (frogs)
		Grahamella (moles)
		Bartonella bacilliformis (verruca peruviana and Oroya fever)
		Bartonella muris (splenectomized rats)
		Protozoan-like bodies in white blood cells of fowls in Palestine and Nigeria
B. NUCLEUS	{	Polyhedral disease of certain caterpillars
		Foot-and-mouth disease
		Vesicular stomatitis
		Borna disease
		Virus III infection of rabbits
		Herpes zoster
		Salivary gland disease of guinea pigs
		Epithelioma of fish
		Carp-pox
		Warts of <i>Discoglossus pictus</i> (frog)
		Warts
		Condyloma acuminatum
		Psoriasis
C. CYTOPLASM AND NUCLEUS	{	Protozoan-like bodies observed in human visceral lesions of unknown etiology
	{	Smallpox (and alastrim)
		Cow-pox (vaccinia)
		Paravaccinia
		Chicken-pox
		Infectious myxomatosis of rabbits
		Symptomatic herpes

selected diseases have been placed in Table II and grouped according to the location of the significant inclusions within affected cells. It is not unlikely that other diseases will be added to the table, and that some now included may in the future be omitted. In Plates 22 and 23 the significant inclusions of the diseases listed in Table II are graphically portrayed.

NATURE OF INCLUSIONS

Various ideas are held concerning the origin and nature of inclusion bodies, and, in a general way, they may be divided into three groups. By some investigators they are considered merely as products of degeneration, but by others they are believed to be the virus itself, while by yet others they are thought of as virus surrounded by a mantle of altered cellular material.

Inclusions as Virus Itself: The idea that inclusions represent the virus itself is not absolutely irrational, inasmuch as bacteria and protozoa are frequently found within cells. Moreover, some protozoa are obligate parasites and multiply only in the cytoplasm or only in the nucleus of suitable host cells, while others reproduce both in the cytoplasm and in the nucleus of such cells. In fact, observations concerning coccidia, malarial parasites, and the more recently described organism of Wright and Craighead have in the main been responsible for the idea that inclusions are parasites.

Inclusions as Virus Surrounded by Altered Cellular Material: Upon the discovery that the etiological agents of the diseases under discussion pass through earthenware filters, a group of workers immediately realized that inclusion bodies probably do not represent virus alone, inasmuch as many inclusions (2-15 microns in diameter) are sufficiently large to render such a possibility unlikely. To adapt theories to facts, von Prowazek then described his hypothesis concerning the nature and development of viruses and their relation to inclusions. According to this worker, extracellular forms of a virus, "elementary bodies," are from 0.25 to 1.0 micron in diameter and are able to pass through filters. Upon entering a cell the "elementary bodies" become "initial bodies" which immediately begin to reproduce by division, thus forming a colony of parasites within its host. The cell then reacts to the presence of these minute organisms around which a mantle of altered cellular material is thrown. In this manner von Prowazek's *Chlamydozoa*, mantled

TABLE II

A List of the Filterable Virus Diseases in which Intracellular Changes are Sufficiently Characteristic to be of Significance. The Diseases are Grouped According to the Location of the Significant Changes within the Cells.

A. CYTOPLASM	{ Mosaic disease of certain plants Sheep-pox Cow-pox (vaccinia) Contagious epithelioma (fowl-pox) Molluscum contagiosum Rabies Lymphocystic disease of fish (no reports on filtration) Infectious myxomatosis of rabbits	Inclusions are acidophilic
B. NUCLEUS	{ Polyhedral disease of certain caterpillars Symptomatic herpes Herpes zoster } No reports on Chicken-pox } filtration. Virus III infection of rabbits Salivary gland disease of guinea pigs Borna disease	
C. CYTOPLASM AND NUCLEUS	{ Smallpox (and alastrim) Paravaccinia (no reports on filtration)	

Rickettsia diseases are not included in the table because the evidence is in favor of the idea that there is a distinct difference between rickettsiae and the inclusions discussed in this paper.

After further study, carp-pox, epithelioma of fish, and warts of *Discoglossus pictus* (frog) may be placed in Group B in view of the inclusions (probably acidophilic) described in nuclei of affected cells.

Evidence is increasing in favor of the idea that the nuclei of cells affected by warts and condyloma acuminatum show certain characteristic changes — basophilic masses or “chromophane” masses of Lipschütz.

Trachoma and inclusion blenorria are omitted from the table awaiting further observations concerning the nature and significance of the cytoplasmic inclusions observed in affected cells.

After further study some of the diseases in the table may be removed or new ones may be added.

animals or inclusion bodies, are formed. The host cell finally ruptures freeing the parasites which again become "elementary bodies." In general, von Prowazek's ideas are in accord with those of Lipschütz, who suggests for the small bodies without mantles the name *Strongyloplasmen*, rounded bits of protoplasm. The ideas of these men are plausible enough, yet in most instances it is difficult either to prove or to disprove conclusively whether they correctly portray the actual facts concerning viruses.

Inclusions as Products of Cellular Degeneration: At present, numerous investigators believe that inclusions do not consist of virus and that at least a major portion of the bodies comprises products of cellular degeneration. These workers frankly admit, however, that in most cases, it is very difficult to establish the fact that the virus is not enveloped by the products of cellular reaction. In one instance only has it been possible to show that active virus is not structurally related to the inclusions characteristic of the disease in which they occur. This was accomplished by Glaser, who found that polyhedral bodies observed in virus diseases of caterpillars can be separated from active incitant and when freed from it are incapable of producing disease in normal larvae.

Although many workers consider inclusions as products of cellular degeneration, there is no unanimity of opinion regarding the manner in which they arise and the cellular constituents they comprise. The nuclear inclusions seen in several diseases, *e. g.*, varicella, herpes, and Virus III infection of rabbits, resemble each other so closely that a differentiation of the diseases one from another by means of the appearance of the inclusions alone is impossible. More specificity, however, is observed concerning cytoplasmic inclusions, inasmuch as no two virus diseases exhibit absolutely identical changes in the cytoplasm of affected cells. The marked degree of specificity displayed by these inclusions is believed by Cowdry and others to be due to the fact that the cytoplasm, by virtue of its composition and position in the cell, may respond more readily and more characteristically to different kinds of stimuli arising either intra- or extracellularly. In spite of the tremendous variations exhibited by inclusions of different diseases, the constancy of their size, form, staining reactions, location in cells, and components such as proteins, fats, and lipoids, in any one disease under similar conditions is very striking. This constancy, however, is by no means dependent upon a

homogeneity of the inclusions, for the majority of them comprise several kinds of constituents. A complete review of the ideas concerning the origin and structure of characteristic inclusions is not possible at the present time. Nevertheless, a few opinions will be cited for the purpose of emphasizing the radical manner in which views of competent workers differ.

*Vaccine Bodies:** Guarnieri (1892) believed vaccine bodies to be protozoa, and, since he thought of them as possessing a peculiar power of devouring the cytoplasm of cells, thus creating a hole within which they lie, gave them the name *Cytoryctes vaccinia*. Although Hückel (1898) considered Guarnieri bodies specific for vaccinia, he believed that they are not the virus itself, but arise entirely within the cytoplasm of affected cells. According to him, under the stimulus of the virus, a portion of the cytoplasm undergoing colloid degeneration near the nucleus becomes cyanophilic (blue-staining). Later the erythrophilic (red-staining) cytoplasm in the immediate neighborhood of the blue mass undergoes hyaline degeneration and separates from the rest of the cell. The two masses, the blue in the center surrounded by the red, are situated within a "hole" in the cytoplasm and constitute a vaccine body. Ewing (1904-05) believes that Guarnieri bodies are altered portions of the cytotreticulum into which nuclear material has diffused. According to Cowdry (1922) these bodies are the result of a stimulation of cells by vaccine virus leading to an increase of a substance present in small amounts in normal cells.

Molluscum Bodies: Molluscum corpuscles were first described in 1841 by Paterson, who thought of them as parasites. In 1892, Macallum (Plate 24, Fig. 5) stated that molluscum bodies are merely migrated plasmosomes or modified chromatin arising as a result of hyperplasia and hyperchromatosis. According to him, one might classify the disease as a neoplasm. Lipschütz (1911) believes that three abnormal substances are found in the cytoplasm of cells affected by molluscum virus; migrated nuclear substance, products of a keratin-like degeneration, and a mass of virus, elementary bodies or *Strongyloplasma hominis* (Plate 22, Fig. 24). In 1918, Sanfelice, using Mann's stain in the study of molluscum bodies, described the following steps in their formation. Normal cells swell. Nucleoli re-

* Weigert, 1874, first described and portrayed inclusions in cells affected by smallpox virus. (Plate 23, Fig. 8.)

tain the red instead of the blue stain and migrate into the cytoplasm where they again stain blue. In the cytoplasm the nucleoli become vacuolated and granular, and, as they enlarge, assume the appearance of typical molluscum bodies filled with fine red-staining granules. More recently, 1927, Goodpasture expressed his ideas concerning molluscum bodies in the following manner: "The bodies are not derived from extruded nucleoli, nor from any formed cytoplasmic constituent. . . . The minute bodies develop about, and later within, cytoplasmic vacuoles which may be regarded as the cellular response to the presence of a living foreign body . . . elementary bodies of Lipschütz. . . ."

*Bollinger Bodies.** Bollinger, in 1873, described in tissues affected by fowl-pox or epithelioma contagiosum a hyperplastic condition of epithelial cells, many of which were greatly swollen, 25 microns in diameter, and contained near their nuclei large bodies with fat-like appearances. These bodies were considered by Bollinger to be parasites. Michaelis (1904) also found evidences of proliferation and swelling of cells in this disease. The inclusions, according to him, are made up of albuminous and fatty substances. Although he was unable to determine whether the disease is parasitic or not, he was, nevertheless, of the opinion that the inclusions themselves do not represent parasites. Borrel (1904), using Loeffler's flagella stain in the study of material from the lesions of fowl-pox, found myriads of minute coccoid bodies about each of which appeared some kind of capsule. Burnet (1906) thought that the small bodies described by Borrel represent the incitant of the disease and are closely associated with the specific inclusions. Ludford and Findlay (1926) state that the inclusions of fowl-pox appear only in epidermal cells, an occurrence which is probably related to the process of keratinization. They state further (1) that the earliest indication of the activity of the virus on cells is the formation in their cytoplasm of small vacuoles, to the periphery of which minute granules adhere, (2) that mitosis† is usually seen in cells containing such vacuoles, (3) that

* Rivolta, 1869, was probably the first investigator to describe inclusions in fowl-pox.

† In Fig. 7, Plate 24, mitosis is taking place, and, although Ludford and Findlay say nothing regarding the matter, one gets the impression from the picture that the virus body has divided also and that a daughter body will go with each daughter cell. This phenomenon has attracted practically no attention in virus diseases of animals, yet it is a well-known fact (Kunkel and others) that inclusions in mosaic diseases of

the vacuoles increase in size, and coincidentally with the enlargement become enclosed by a lipoidal lining and exhibit a granular appearance internally, (4) that many cells become hypertrophied and at an early stage in the disease show a complete reversal of the Golgi apparatus, and finally (5) that the inclusions are not the virus itself. (See Plate 24, Figs. 6-9.)

Negri Bodies: In nerve cells injured by the virus of rabies Negri (1903) discovered certain inclusions which he regarded as protozoa. Levaditi and his coworkers (1926) still believe in the parasitic nature of Negri bodies and propose that they be named *Glugea lyssae*. According to Acton and Harvey, however, these structures are not parasitic, but arise as a result of an interaction between the cytoplasm of cells and particles of nuclear matter which have been extruded through catabolic changes induced by the action of the virus. In Goodpasture's opinion (1925), Negri bodies represent the results of a slow necrobiosis of nerve cells and originate in a degenerative change in mitochondria producing vacuoles with small bodies within them, "about which through a partial disintegration of neurofibrillar material a capsule is formed."

Nuclear Inclusions: Ideas of the nature and origin of acidophilic nuclear inclusions have also led to numerous discussions. Loewenthal believes that they are parasites. Lipschütz, Goodpasture, and others are of the opinion that the bodies, although not necessarily consisting entirely of virus, are in some manner intimately associated with it. Luger and Lauda, however, contend that these nuclear changes are due to oxychromatic degeneration (Plate 22, Figs. 8-15) and have no genetic affinity with the virus.

Sufficient examples have been cited to show that able investigators frequently disagree radically in their views concerning the inclusions of each virus disease. Furthermore, from what has been said it is obvious that the intracellular structures described in various morbid conditions may not be of a similar character. Therefore, in discussing intracellular pathology, one should be careful not to make general statements based on observations limited to one virus

certain plants frequently divide when the host cells do and that a daughter inclusion body goes with each daughter cell. This phenomenon is interesting, but it does not necessarily imply that the inclusion is an autonomous parasite, inasmuch as the Golgi apparatus and at times mitochondria (Cowdry) divide during cell division, and a portion of each structure goes with each daughter cell.

TABLE III

A List Indicating Hypotheses Concerning the Nature and Origin of Inclusions Observed in Cells Affected by Viruses

A. CYTOPLASMIC INCLUSIONS

1. Bacteria.
2. Fungi.
3. Protozoa.
4. Products of cellular degeneration or cellular reaction to the viruses. According to Lipschütz and others, the etiological agents are visible; they are called stronglyplasms, initial bodies, or elementary bodies. According to Prowazek and others, inclusion bodies consist of viruses surrounded by products of cellular reaction; they are called chlamydozoa (mantled animals).
5. Products of cellular degeneration; agent unseen or not identified; no proof of genetic relation between inclusion and virus.
 - (a) Engulfed or phagocytosed cells undergoing degeneration.
 - (b) Nucleoli or necrotic nuclear derivatives extruded into the cytoplasm.
 - (c) Extruded nuclear material mixed with cytoplasmic elements.
 - (d) Central body or archoplasmic structures undergoing degeneration.
 - (e) Degeneration of cytoplasm and cytoplasmic structures.
 - (f) Degeneration of daughter nuclei in cells in which amitotic division of the nuclei occurs.
 - (g) Results of secretory activity of affected cells.
 - (h) Inclusions consist of substances normally present in small amounts in the cytoplasm. Under the influence of viruses, however, these substances are genetically increased.

B. NUCLEAR INCLUSIONS

1. Protozoa.
2. Similar to No. 4 under Cytoplasmic Inclusions.
3. Nucleoli undergoing degeneration.
4. Results of nuclear degeneration (oxychromatic degeneration). Inclusions are not genetically related to the viruses.
5. Similar to (h) under No. 5 (Cytoplasmic Inclusions) except that the changes occur in the nucleus instead of in the cytoplasm.

disease. For convenience, a summary of the ideas regarding the mode of origin and the nature of inclusions is given in Table III.

CHANGES PRODUCED IN INCLUSIONS BY EXPERIMENTAL PROCEDURES

The relation of smallpox to vaccinia furnishes an interesting topic for discussion. In most respects these diseases behave differently: one is highly contagious, the other is spread only by direct inoculation; in one, generalized lesions regularly occur, in the other, this

happens only rarely; in cells affected by smallpox, inclusions are seen in the nucleus as well as in the cytoplasm, in cells injured by vaccinia, significant inclusions are found only in the cytoplasm. Smallpox virus, however, passed through several calves, becomes vaccine virus. Furthermore, it remains vaccine virus, even though it be returned to human beings. Thus, smallpox virus passed from man to lower animals, excepting monkeys, is so altered that apparently a different disease is caused by its action. Not only is the character of the disease altered but the intracellular pathology is also changed. Observations of a similar nature have been made in regard to rabies. When "street virus" is passed through a large number of rabbits a "fixed virus" is obtained which no longer produces typical inclusions but causes atypical Negri, lyssa, or passage bodies. These facts are extremely interesting, and certainly, so far as smallpox and vaccinia are concerned, no one has been able to determine just what occurs when smallpox virus becomes vaccine virus. Nor is it known why the intracellular pathology under these conditions is also altered.

INCLUSIONS IN MALIGNANT GROWTHS

Paterson saw molluscum bodies in 1841, Bollinger described inclusions in contagious epithelioma in 1873, and Guarnieri observed vaccine bodies in 1892. In spite of these facts, the chief interest in inclusion bodies during the latter part of the nineteenth and the first part of the twentieth centuries centered around peculiar intracellular structures observed in cells of neoplasms. Some of this interest may have been due to the fact that many workers considered molluscum contagiosum and epithelioma contagiosum as tumors. Aside from that, however, great interest was evidenced regarding the so-called cancer inclusions described by Plimmer, Feinberg, von Leyden, Russell, Schüller, Thoma, Darier, and others.

Practically all inclusions described in tumor cells have been situated in the cytoplasm. Many of them have been portrayed as having definite and characteristic structures, *e. g.*, Plimmer bodies. Hypotheses regarding the nature of cancer inclusions are numerous, and strange to say, with a few exceptions, are similar to the theories in Table III concerning cytoplasmic inclusions in virus diseases. Interest in these structures in cancer resulted in little or no progress in the recognition of the cause of newgrowths. Furthermore, the

irregularity of their occurrence, and the difficulty encountered in finding them, rendered these bodies of little value as a diagnostic aid. Consequently, cancer inclusions are rarely mentioned at the present time.

Concurrently with a decreasing interest in cancer bodies and a better understanding of the nature of such diseases as contagious epithelioma and molluscum contagiosum the inclusions in virus diseases received an increasing amount of attention. The regularity with which characteristic structures are found in cells affected by certain morbid conditions render them of value in experimental and diagnostic work, *e. g.*, in rabies and in smallpox. Jackson and Goodpasture's descriptions of nuclear changes observed in duct cells of guinea pigs' salivary glands induced Cole and Kuttner to search for a virus. Their investigations resulted in the discovery of a new filterable virus which injected into susceptible pigs produces nuclear changes identical with those described by Jackson and Goodpasture. Moreover, all work with polyhedral diseases of caterpillars is controlled by the presence or the absence in blood cells of characteristic nuclear changes. Consequently, it is unlikely that the inclusions in virus diseases will cease to be of interest as quickly as did those of tumors. It is true that the nature of the intracellular bodies has not been definitely determined. Nevertheless, in spite of the ignorance concerning their nature, inclusions have held and will continue to hold an important position in the study of this group of diseases.

Many attempts to produce significant inclusions by artificial means have been unsuccessful. It is interesting, however, to note that, when intracellular changes resembling inclusions of virus diseases have been experimentally induced, they have followed the use of such agents as arsenic, tetanus toxin, and diphtheria toxin. Some of the experimentally induced changes closely resemble those seen in virus diseases, yet they are not identical. Therefore, under properly controlled conditions, the presence of inclusions, accepted as significant, will undoubtedly in the majority of instances be indicative of the presence of a virus in the immediate vicinity.

PATHOLOGICAL CHANGES OTHER THAN INCLUSION BODIES

In the foregoing section of the paper, facts and conjectures regarding inclusion bodies have been discussed. While these intracellular structures play an important rôle in the study of virus diseases, they, nevertheless, constitute a minor part of the reaction in the host to the infectious agents. Furthermore, if no inclusions are found in cells affected by certain disease processes, or if the intracellular changes described have not been generally accepted as significant, one is not warranted in concluding that the morbid conditions are not caused by viruses. This statement is emphasized by the fact that significant inclusions have not been described in measles, poliomyelitis, and fowl plague, diseases generally believed to be caused by filterable viruses. Therefore, one is justified in raising the question as to whether inclusions constitute the only characteristic response of cells to viruses. Moreover, one would like to know whether, in addition to specific or characteristic responses, there are also reactions similar to those induced by substances of bacterial or other origin.

INFLAMMATION

Although the majority of pathologists interested in virus diseases have devoted most of their energy to the study of inclusions, there is a group of workers who either ignore the existence of such structures or believe that they are of no significance, and contend that the pathology of certain virus diseases is not essentially different from that observed in inflammatory processes produced by ordinary bacteria. Furthermore, as one reads the reports of work recorded in the literature, one is impressed by the fact that many investigators assume that inflammation caused by bacteria is thoroughly understood. Of course, such is not the case, inasmuch as it is not known whether certain responses of the host are due to the direct action of bacteria and their products, whether they are dependent upon the presence of substances derived from host cells that have been injured or killed by bacterial activity, or whether they are caused by the action of an injurious agent formed or liberated when infectious organisms (antigen) unite with their specific antibody.

The fact that inflammation occurs in many virus diseases cannot be denied, and, despite the acute nature of some of the diseases, if

secondary infections do not intervene, the inflammatory process is usually characterized by an infiltration of mononuclear cells. The question whether the inflammation is a primary or secondary phenomenon, however, has in certain instances led to lengthy discussions. Weigert (1874) looked upon the primary changes caused by the virus of smallpox as non-inflammatory and considered them to be necrobiotic or diphtheroid in nature. According to him, when the primary degenerative changes in the epidermis have reached a certain stage in their development, the inflammatory reaction appears as a secondary phenomenon and consists of exudation of fluid into the area of degenerated cells, of proliferation of epidermal cells with giant-cell formation around the necrotic tissue, and of infiltration of various kinds of leucocytes. Auspitz, Unna, Renaut, Ledingham, and others have regarded the variolous changes in the skin from the beginning as merely the expression of an acute inflammatory process. Furthermore, Unna and Ledingham have raised the question whether the virus of smallpox must necessarily cause necrosis of epidermal cells and whether the necrobiosis frequently observed is only accidental or secondary to an inflammatory reaction which begins in the corium. According to this view, the degenerative changes in epidermal cells and the formation of pocks result from pressure caused by the accumulation of exudate just beneath the epidermis.

In a number of diseases, particularly those involving the skin, it is difficult to determine precisely the location and the character of the primary injury or to follow accurately the development of a lesion. This difficulty is experienced because of the complex nature of the skin and the rapidity with which the lesions of such diseases as measles, smallpox, and varicella develop. If the inciting agent is borne into the skin by the blood, it is highly probable that the initial injury is in or around small blood vessels just beneath the epidermis, provided the cells in these tissues are susceptible to the virus.

So far as the discussion in the present paper is concerned, it is not so important to determine the point of the primary lesion caused by the virus as it is to ascertain the nature of the injury. Frequently conditions are unfavorable for such investigations. In some diseases, however, and under certain conditions in others, information concerning this question may be obtained. For instance, in variolous or vaccinal lesions of a rabbit's cornea definite and characteristic

changes are observed in the epithelial cells before any evidence of inflammation in the form of cellular or other exudate is seen. The lesions of mollusum contagiosum occur within the epidermis, and according to Benda,* Unna,* and Goodpasture (1927) little or no inflammatory reaction is observed in the corium. Furthermore, if inflammation occurs accidentally through secondary infection or as the result of treatment, the lesions heal promptly (Henderson, 1841). Goodpasture (1925) working under experimental conditions, found that the first evidences of injury caused by rabic virus are observed "within ganglion cells, not in the surrounding tissue," and that "these cells may undergo complete necrosis without cellular or other exudate about them." He also found that Negri bodies frequently "occur in great numbers within the ganglion cells entirely in the absence of evidences of inflammation in the form of cellular or other exudation." Finally, the pathological picture presented by mosaic disease in plants is one of necrosis and hyperplasia, and that observed in bacteriophagy is unlike the morbid processes usually associated with inflammatory diseases.

DEGENERATION AND PROLIFERATION

If inflammation, as it appears to be, is a secondary phenomenon in many virus diseases, what, then, are the primary changes produced in cells by these active agents? In all probability, they are either degenerative or proliferative in character. In fact, both types of changes are usually seen, and it is difficult at times to determine definitely whether degeneration precedes proliferation or whether they occur in the reverse order.

In certain diseases, particularly the ones producing vesicles in the skin, the degenerative changes have attracted most attention and the majority of workers consider that they precede evidences of proliferation. Opinions as to the type of degeneration, however, are numerous and many names have been used in its description, *e. g.*, coagulation necrosis, reticulating colliquation, and diphtheroid, fibrinoid, colloid, hyaline, parenchymatous, ballooning, or reticulating degeneration. Be that as it may, the type of degeneration in certain virus diseases is somewhat different from that ordinarily observed, inasmuch as the "reticulum" in the vesicles is formed by the

* Cited by White and Robey.

remains of swollen degenerated cells that have lost their nuclei and from which the major portion of the cytoplasm has disappeared (Plate 26, Figs. 1-8).

To investigate the relation of degeneration to proliferation in tissues infected with vaccine virus or smallpox virus, one should observe the phenomena as they occur in the cornea of rabbits. Within a short time after the cornea is inoculated, 3 to 6 hours, changes are seen in the immediate vicinity of the point infected; the epithelial cells are larger and stain less intensely than usual, mitotic figures and amitotic giant cells begin to appear. Within 6 to 24 hours, vaccine bodies are frequently found in affected cells. Small, yet macroscopic nodules are observed on the surface of the cornea 24 to 48 hours after inoculation. Examination of these nodules reveals, in addition to a hypertrophy of individual cells, a definite increase in the number of cells as compared with the findings in control areas. At this time, 48 hours after inoculation, evidences of degeneration and inflammation appear. Guarnieri, in 1892, von Wasielewski, in 1901, and Paul, in 1916, graphically recorded many of the changes just described (Plate 25, Figs. 1-4). Moreover, von Wasielewski stated that the first influence of vaccine virus is to produce increased nutrition and enlargement of cells. Therefore, as far as vaccinal and variolous infections in cornea of rabbits are concerned, one seems justified in concluding that the pathological changes occur in the following order: (1) stimulation and proliferation, (2) degeneration, (3) inflammation.

At this point it is interesting to note that in sheep-pox, a disease similar to vaccinia or cow-pox, Bosc and others have described remarkable proliferative changes in the skin, lungs, and other organs of the body. The proliferation in the lungs (Plate 25, Fig. 5) is evidenced by minute translucent nodules, "sheep-pox adenomas," which represent alveoli completely filled with peculiar, pale-staining cells with vesicular nuclei. Bosc believed the cytoplasmic inclusions (Plate 22, Fig. 22) observed in sheep-pox to be parasites, and, in view of the fact that hypertrophy and hyperplasia of cells are quite evident in infected tissues, he proposed for the inciting organism the name *bryocyte*, an agent causing cells to proliferate.

Mosaic disease in certain plants, frequently spoken of in Germany as "Pockenkrankheit," resembles in many respects the vesicular eruptions observed in animals. This similarity is particularly notice-

able when fruits are attacked (Plate 27, Figs. 1-8). The pathological picture presented by mosaic is said to be characterized by necrosis and hyperplasia occurring in the order mentioned. In view of the fact, however, that mosaic inclusion bodies are found in living cells, some of which are undergoing division, and in the light of what is known concerning other virus diseases, it is possible that further studies concerning the relation of necrosis to hyperplasia in mosaic conditions may reveal some interesting facts.

In *molluscum contagiosum* the pathological changes in the epidermal cells are evidenced by hypertrophy and hyperplasia (Macallum) which are followed by degenerative activities (Plate 24, Fig. 5). Somewhat the same course of events is observed in warts and *condyloma acuminatum*. Contagious epithelioma of fowls, or fowl-pox, is a disease with two names, each of which suggests a pathological process different from that indicated by the other. At one time this morbid condition was believed to be neoplastic in nature, but at present the tendency is to consider it closely allied to other pock diseases. In spite of the presence of warty growths that characterize the disease, one should remember that a destruction of tissue with vesicle formation also occurs (Plate 26, Fig. 8).

In lesions of infectious myxomatosis of rabbits first described by Sanarelli, the subepidermal tumor-like masses have attracted most attention. Interesting changes, however, have been observed also in the epidermis where a marked swelling and then a complete dissolution of the cells take place. In this disease it appears that proliferative phenomena predominate in the corium and subcutaneous tissues to such an extent that tumor-like masses are formed, while in the epidermis retrograde changes with vesicle formation prevail (Plate 26, Figs. 1-3).

The virus of vesicular stomatitis injected in the pads of guinea pigs causes rapid destruction of epithelial cells, and within 18 to 24 hours after inoculation, vesicles are already well developed. While at this time many swollen cells are still present in the lesions, mitotic figures, amitotic giant cells, and other evidences of proliferation are either absent or present in small numbers (Plate 26, Fig. 7). I have not had the opportunity of examining lesions earlier than 18 hours after inoculation, consequently I do not know whether an appreciable amount of proliferation precedes so rapid a destruction of cells as that observed in this disease. In the light of what is now known con-

cerning the bacteriophage, it is conceivable, however, that such a proliferation may occur.

The lysis* of bacteria by bacteriophage, as the name applied to the phenomenon suggests, has been considered the most important feature of the action of this agent on microorganisms. D'Herelle, Hadley, and others, however, have spoken of an initial stimulation of bacteria as evidenced by an increase in rate of their multiplication. It has also been observed repeatedly that the size of individual cells increases considerably under the influence of phage. The constancy or the importance of this swelling as a factor in the disappearance of bacteria has not been generally accepted. Moreover, it has been suggested that swollen bacteria are very resistant to dissolution and disappear, if at all, very slowly.

The extent of swelling of individual bacteria, the relative proportion of swollen cells, as well as the actual relation between the swelling and the lysis, are difficult to establish by the usual methods of observation. Consequently, Dr. Bronfenbrenner, with the assistance of Mr. Rosenberger, investigated the matter cinematographically. The following is a brief description of their findings: After a short period of lag, bacilli under the influence of phage began to multiply at a rate noticeably exceeding that of normal organisms photographed under similar conditions. Many cells failed to complete their division and filaments having a length of from 10 to 20 times that of normal bacteria were frequently observed. By the end of the first hour of growth, occasional cells had already begun to swell, and by the end of the third hour the majority of bacteria in the field appeared more or less swollen. The swelling continued slowly until about the fifth hour, when, one by one, the bacteria suddenly and quickly disappeared, leaving little, if any, evidence of their former existence (Plate 28, Figs. 1-3).

Bronfenbrenner has also shown that in stained preparations the cytoplasm of swollen bacteria takes the dye less intensely and less evenly than does the cytoplasm of normal cells, in consequence of which it frequently appears segmented or beaded (Plate 28, Fig. 2). These observations were substantiated by photographs of unstained bacteria made by means of ultra-violet light. The rapid melting

* The description of the pathological picture observed in bacteria undergoing lysis is taken from Bronfenbrenner, Muckenfuss, and Hetler's paper, "The study of intimate mechanism of the lysis of bacteria by bacteriophage," *Am. J. Path.*, 1927, iii, 562.

away of the bacteria recorded cinematographically together with observations on the appearance of swollen bacteria in stained and unstained preparations probably indicates that the cytoplasm of bacteria under the influence of phage is liquefied within the cells prior to the disappearance of their membranes.

From the observations recorded it seems that in lesions produced by the majority of viruses, phenomena related to stimulation, proliferation, and degeneration of cells, as well as those connected with inflammation, occur. In some cases, all of the phenomena do not appear, because under the existing conditions it is impossible for certain of them to take place. For instance, in diseases that attack nerve cells, as in rabies, no proliferation of the involved cells has been described. In such diseases the first response of the affected cells is probably an alteration in their metabolism or a necrobiosis. Nor does inflammation in the form of cellular exudate always occur, either because certain tissues are involved, *e. g.*, the epidermis in molluscum contagiosum, or because certain hosts, *e. g.*, plants and bacteria, cannot respond to injury in this manner. Finally, in diseases in which all the phenomena occur, one frequently experiences difficulty in determining the order of their occurrence. In some diseases, however, and under certain conditions in others, the pathological changes apparently take place in the following order: (1) hypertrophy and hyperplasia,* (2) degeneration or necrobiosis, and (3) inflammation in the form of cellular or other exudation.

RELATION OF PROLIFERATION, DEGENERATION, AND INFLAMMATION TO THE FORMATION OF INCLUSION BODIES

Inasmuch as typical inclusion bodies are frequently observed in cells before any evidences of inflammation in the immediate neighborhood are discernible, it appears that these structures are not directly related to the products of inflammation, *e. g.*, engulfed leucocytes.

Although the question is still open as to whether the incitants of many virus diseases are structurally related to their significant inclusions (enveloped by them), and in spite of the contention of certain workers that inclusions represent parasites, it seems most likely

* Some workers may contend that these changes are the first responses to many kinds of injury and constitute evidences of degeneration. Be that as it may, in any event they are evidences of stimulation and proliferation.

that at least the major portion of the constituents of these peculiar bodies are in some manner related to the phenomena of proliferation and degeneration of host cells. Furthermore, in the majority of instances they appear to be more closely connected with hypertrophy and hyperplasia of cells than with retrograde changes. In rabies, a disease in which little or no proliferation of affected cells occurs, degenerative processes or altered cell metabolism may be responsible for the formation of inclusions. In morbid conditions, however, where proliferation precedes, or at least goes hand in hand with degeneration, and where inclusions are found in actively growing and dividing cells (Plate 22, Figs. 1 and 3; Plate 23, Figs. 1 and 8; Plate 24, Fig. 7) it seems not unlikely that the cellular activity induced by the viruses may lead to the formation of the inclusions either through the overproduction or the modification of some substance or substances normally present in cells, or in consequence of the retention of material ordinarily excreted. These views receive further support from observations concerning the size of cells affected by viruses. In certain instances their diameter increases from 7 to 400 microns (Plate 24, Figs. 1-4). Such an enhancement of size forces one to admit that the changes giving rise to it cannot result from degeneration alone and can take place only in living cells with a fairly active metabolism.

According to these views, inclusion bodies constitute the visible manifestations of a series of activities taking place in living and frequently in growing cells under the stimulating and degrading influences of certain viruses. In most instances the structural relation of the incitants to their specific inclusions is an open question. The distinctive differences observed in inclusions may be dependent upon the species of host, the type of cell and its portion affected, and the nature of the stimulus in the form of virus or its activity. It is true that proliferation and degeneration of cells are observed in some virus diseases and also in diseases other than those caused by viruses without the occurrence of typical inclusions in affected cells. The nature of the stimulus which determines the type and extent of changes taking place in these diseases probably accounts for the absence of inclusions under these conditions.

SUMMARY

There are diseases caused by certain peculiar incitants, viruses, that produce in their hosts pathological changes not entirely unlike those found in other diseases, yet sufficiently different from them in regard to phenomena related to proliferation and degeneration to warrant placing such agents in a group by themselves. If proliferative phenomena predominate, pathological conditions such as warts, molluscum contagiosum, and tumors result. If destructive or retrograde changes prevail, diseases such as varicella, vesicular stomatitis, and lysis of bacteria are the consequence.

Cells affected by many of the diseases in the group exhibit intracellular changes sufficiently characteristic to be spoken of as inclusion bodies. These structures are probably closely related to the proliferative and degenerative phenomena induced in cells by the action of viruses.

The views expressed in the present paper concerning the pathological conditions observed in virus disease are consistent with living or with lifeless incitants multiplying either intra- or extracellularly. The pathological changes, however, as well as other phenomena, emphasize the fact that in virus diseases an intimate type of parasitism exists.

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DESCRIPTION OF PLATES

PLATE 22

- FIGS. 1 and 2. Epithelial cells affected by the virus of contagious epithelioma; the pink-staining masses in the cytoplasm represent Bollinger bodies. Fig. 1 also shows the result of amitotic division of the nucleus. Zenker's fixative; eosin and methylene blue stain. $\times 1700$.
- FIGS. 3 and 4. Cells, probably endothelial leucocytes, in the interstitial tissue of a rabbit's testicle affected by Virus III. The inclusions are represented by the pink intranuclear masses. Fig. 3 represents an amitotic giant cell. Zenker's fixative; eosin and methylene blue stain. $\times 1700$.
- FIGS. 5-7. Human epithelial cells injured by the virus of varicella. The pink masses in the nuclei are the characteristic inclusions. Fig. 7 represents an amitotic giant cell. Zenker's fixative; eosin and methylene blue stain. $\times 1700$.
- FIGS. 8-11. These figures represent a, b, e, and g respectively of Abb. 8 in Lauda and Luger's article on herpes simplex, *Ergbn. inn. Med. u. Kinderheilk.*, 1926, xxx, 377, and show the initial stages of oxychromatic nuclear degeneration.
- FIGS. 12 and 13. These figures represent a and b respectively of Abb. 9 in Lauda and Luger's article on herpes simplex (see above), and show oxychromatic nuclear degeneration fully developed.
- FIGS. 14 and 15. These figures represent 2 cells of Abb. 10 in Lauda and Luger's article on herpes simplex (see above), and show the end stages of oxychromatic nuclear degeneration.
- FIGS. 16 and 17. Vaccine bodies — Guarnieri bodies — in the cytoplasm of corneal epithelial cells of a rabbit, 48 hours post inoculation. Zenker's fixative; eosin and methylene blue stain. $\times 1700$.
- FIG. 18. Represents one cell from Abb. 18 of Lipschütz's article on the herpes group of diseases, *Arch. Dermatol. u. Syph., Orig.*, 1921, cxxxvi, 428. The cell, corneal epithelial cell of a rabbit inoculated with herpes simplex virus, shows an acidophilic nuclear inclusion. Sublimate alcohol fixation; hämalaun eosin stain; Zeiss 1/12 im. and oc. 4.
- FIG. 19. Represents an amitotic giant cell in human genital herpes. From Abb. 10 in Lipschütz's article (see above). Sublimate alcohol fixation; Giemsa's stain; Zeiss 1/12 im. and oc. 4.
- FIG. 20. Represents a human epithelial cell affected by the virus of herpes zoster. From Abb. 1 in Lipschütz's article (see above). The characteristic change is the acidophilic mass in the nucleus. Sublimate alcohol fixation; Giemsa's stain; Zeiss im. and oc. 4.

FIG. 21. Reproduction of a cell from Fig. 5, Plate V, of Negri's article on rabies, *Z. Hyg., u. Infektionskrankh.*, 1903, xliii, 507, and shows a cell from Ammon's horn of a dog that died of rabies 15 days post infection. In the cytoplasm is a typical Negri body. Zenker's fixative; Mann's stain; Zeiss 2 mm. and oc. 6.

FIG. 22. Reproduction of a cell from Fig. 15, Plate II, of Bosc's article on sheep-pox, *Centr. Bakt., 1. Abt., Orig.*, 1903, xxxiv, 413, 517, 666. It is an epithelial cell with a cytoplasmic inclusion and vacuolated nucleus. Flemming's fixative; magenta phenol picro-indigo-carmin stain; Zeiss 1/12 in.

FIG. 23. Represents Fig. 5 (slightly modified) of Plate XXVIII illustrating Weissenberg's article on lymphocystic disease of fish, von Prowazek's *Handbuch der pathogenen Protozoen*, 1921, Leif. 9, 1344. The cell, 27 microns in diameter, shows several cytoplasmic inclusions. Acetic alcohol fixation; hematoxylin and Biondi stain. $\times 1025$.

FIG. 24. Reproduction from Plate XVI illustrating Lipschütz's article on molluscum contagiosum, *Arch. Dermatol. u. Syph.*, 1911, cvii, 387, represents an epithelial cell with its nucleus compressed and pushed to the periphery and with its cytoplasm practically replaced by a so-called molluscum body. Sublimate alcohol fixation; Giemsa's stain; Leitz im. 1/12 and oc. 8.

FIG. 25. Epithelial cell of rabbit affected by the virus of infectious myxomatosis. The cell is swollen, the nucleus is vacuolated, and the cytoplasm contains a large granular pink-staining mass in the midst of which are several blue coccoid bodies. Zenker's fixative; eosin and methylene blue stain. $\times 1700$.

FIG. 26. Reproduction of Fig. 5, Plate II, illustrating Joest's chapter on Borna disease in Kolle and von Wassermann's *Handbuch der pathogenen Mikroorganismen*, Jena, Fischer, 2nd ed., 1913, vi, 251. Ganglion cell from Ammon's horn showing pink nuclear inclusions. Lentz's stain; Zeiss im. 1/12 and oc. 3.

FIG. 27. Reproduction of one cell from Fig. 1, Plate 33, illustrating Cole and Kuttner's article on salivary gland virus of guinea pigs in *J. Exp. Med.*, 1926, xlv, 855. It is a swollen duct cell with eosin-staining nuclear inclusion from a submaxillary gland of a full grown pig. Zenker's fixative; eosin and methylene blue stain. $\times 1700$.

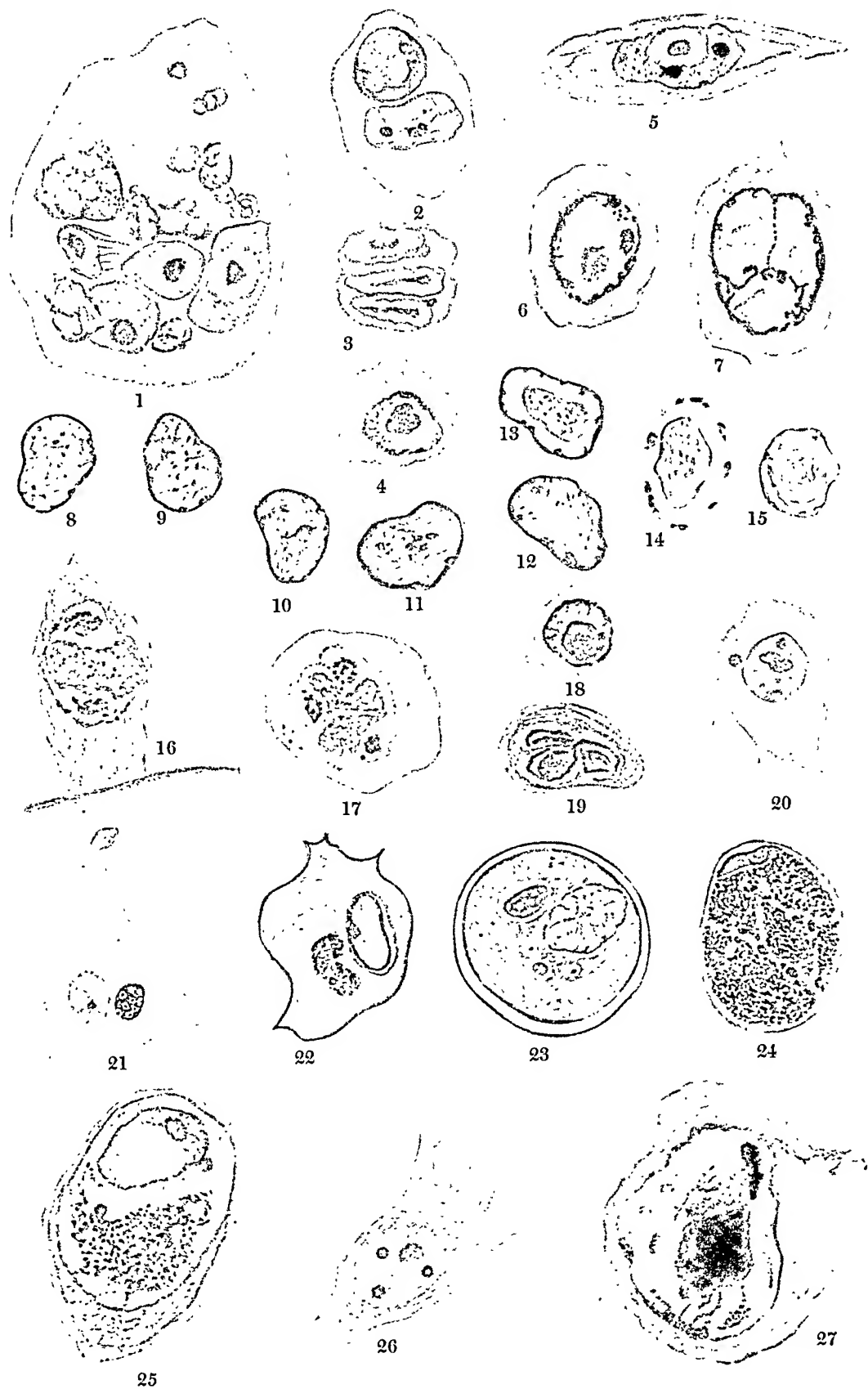


PLATE 23

- FIG. 1. Tobacco mosaic. "Young palisade cell containing striate material radiating from the nucleus and a crescent-shaped body (A) adjacent to nucleus." (After Rawlins and Johnson, *Ann. J. Bot.*, 1925, xii, 19.)
- FIG. 2. "Leaf cell containing mitotic figure, crescent-shaped body (A), and striate material." (After Rawlins and Johnson.)
- FIG. 3. "Cell of a short glandular trichome containing a body (A) of the same type shown in Fig. 4." (After Rawlins and Johnson.)
- FIG. 4. "Cell of a short glandular trichome showing striate material and vacuolate body (A) containing a dark-staining granule." (After Rawlins and Johnson.)
- FIG. 5. Intracellular body (marked X) in rosette-diseased wheat. (After McKinney, Eckerson, and Webb, *J. Agric. Res.*, 1923, xxvi, 605.)
- FIG. 6. Shows nuclear and cytoplasmic inclusions in paravaccinia. Reproduction of Fig. 12, Plate III, in Lipschütz's article on paravaccinia, *Arch. Dermatol. u. Syph.*, *Orig.*, 1919-20, cxxvii, 193.
- FIG. 7. Reproduction from Guarnieri's article on vaccinia in 1892 showing a vaccine body in the cytoplasm.
- FIG. 8. Reproduction from Weigert's article on smallpox in 1874 showing amitotic giant cells and bodies in cytoplasm of affected cells similar to the ones later described by Guarnieri.
- FIGS. 9-12. Cells showing nuclear and cytoplasmic inclusions in smallpox. (After Councilman, Magrath, and Brinckerhoff, *J. Med. Res.*, 1904, xi, 12.)
- FIGS. 13 and 14. Polyhedral bodies in jaundice of silkworms. (After von Prowazek, *Centr. Bakt.*, 1. *Abl.*, *Orig.*, 1912-13, lxvii, 268.)

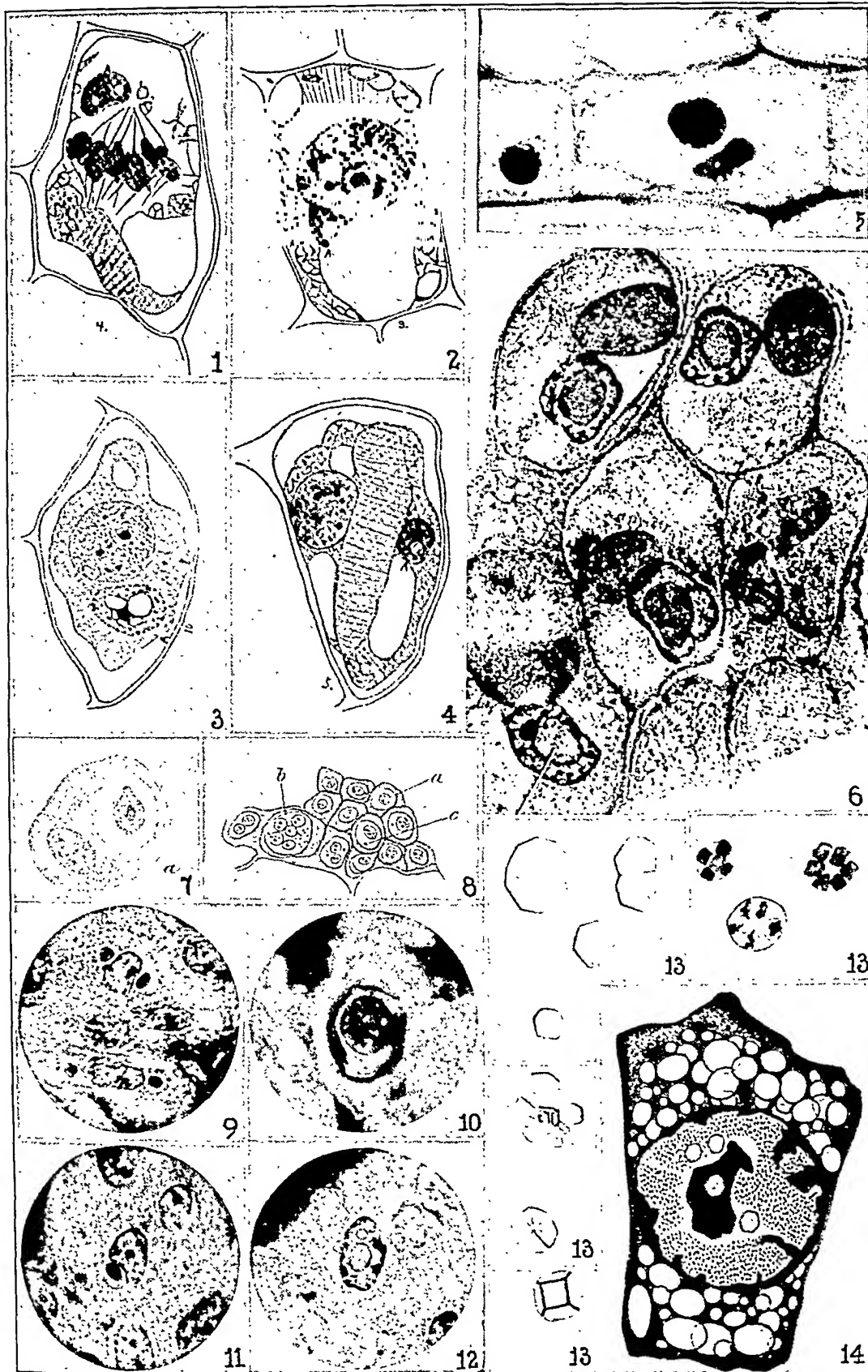


PLATE 24

FIGS. 1-4. Illustrations by Joseph (1918) showing the remarkable increase in size of cells affected by the virus of lymphocystic disease of fish. In Fig. 1, there are three small dark cells showing the early effect of the virus, and two large ones exhibiting the picture presented at a later stage. Fig. 3 presents a cell going to pieces. Fig. 4 shows new cells filling the space left by one degenerated cell. Fig. 1 = $\times 250$; Fig. 2 = $\times 650$; Fig. 3 = $\times 200$; Fig. 4 = $\times 200$.

FIG. 5. Reproduction from Macallum's (1892) article showing the changes that take place in epidermal cells under the influence of molluscum contagiosum virus.

FIGS. 6-9. Reproductions from Ludford and Findlay's article (1926) on fowl-pox. Fig. 6 shows small vacuole with minute adherent granules. Fig. 7 represents a cell with a mitotic figure and two virus bodies. Fig. 8 shows reversal of Golgi apparatus. Fig. 9 presents a well developed virus body in a greatly swollen cell.

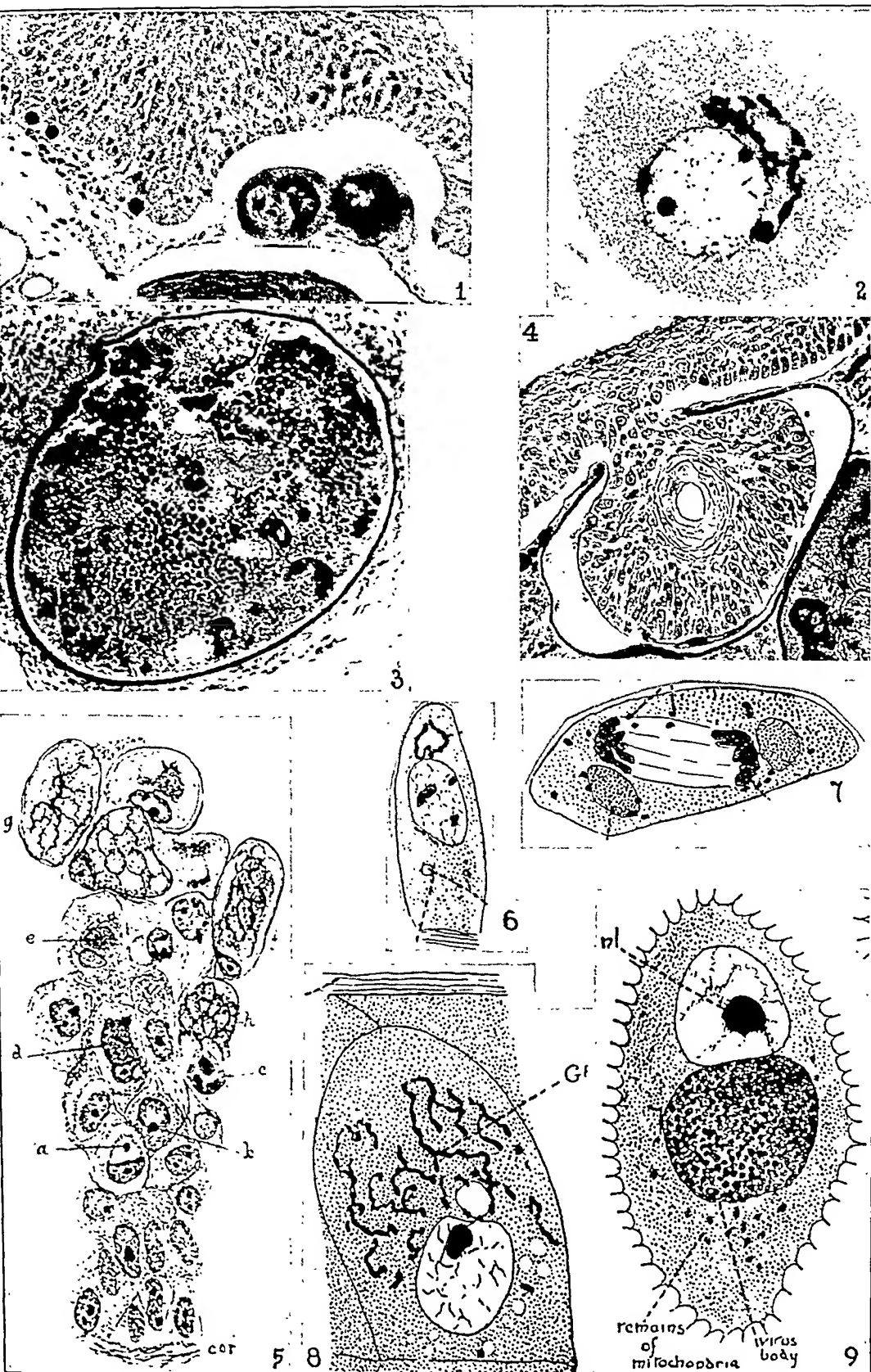


PLATE 25

FIG. 1. Reproduction of illustration by Guarnieri (1892) showing hyperplasia and hypertrophy of corneal cells with vaccine bodies caused by the action of vaccine virus.

FIG. 2. Reproduction of illustration by von Wasielewski (1901) showing hyperplasia and hypertrophy of corneal cells with amitotic giant-cell formation caused by the action of vaccine virus.

FIG. 3. Illustration by Paul (1916) showing the effect of smallpox virus on the corneal cells of a rabbit 86 hours after inoculation.

FIG. 4. Illustration by Paul (1916) showing hyperplasia and hypertrophy of corneal cells caused by smallpox virus 48 hours after inoculation. No evidence of inflammation at this time.

FIG. 5. "Sheep-pox adenoma" of lungs. (After Bosc, 1903.)

FIG. 6. Hyperplasia and hypertrophy of cells in the skin caused by the virus of sheep-pox. (After Bosc, 1903.)

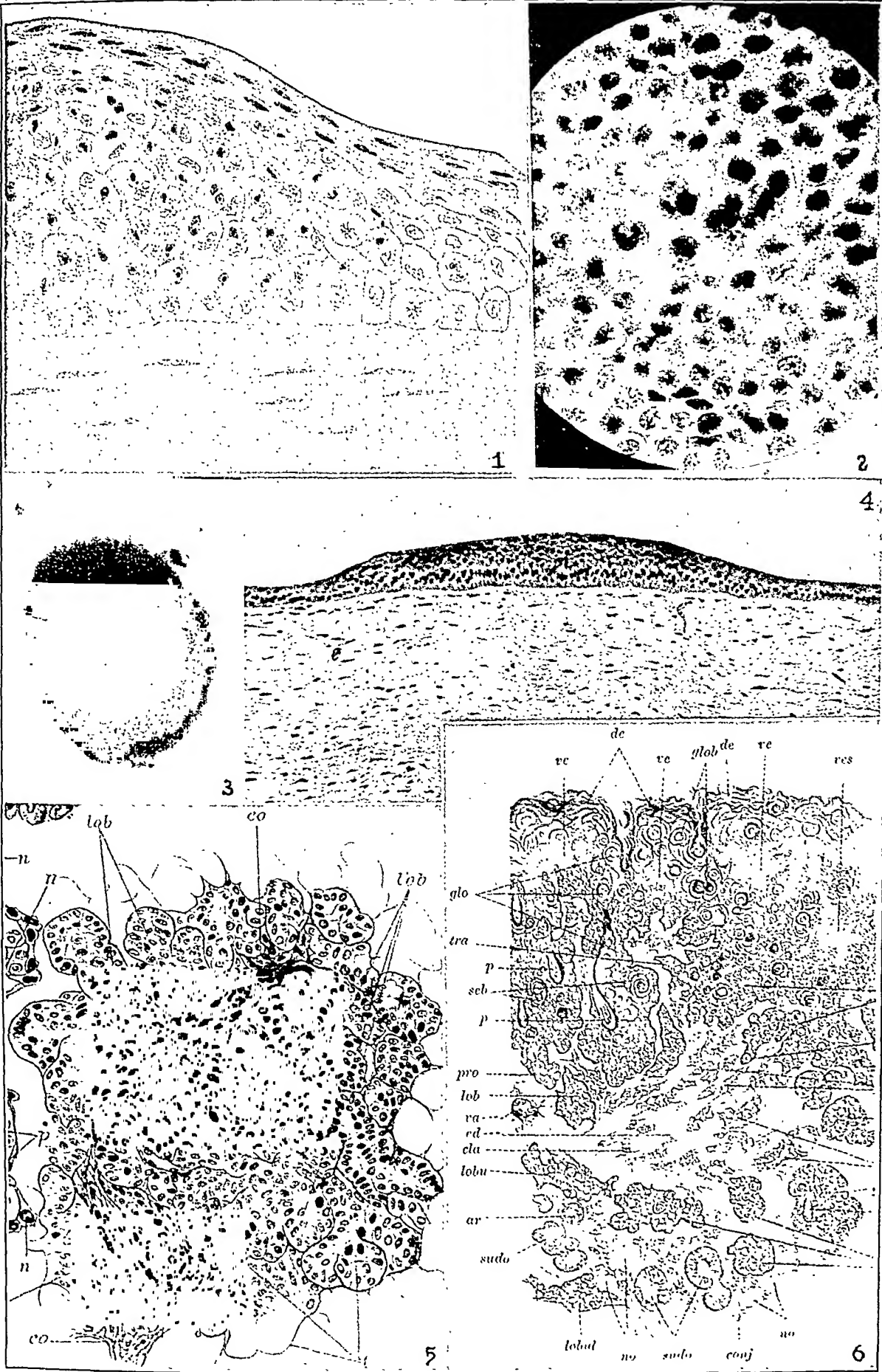
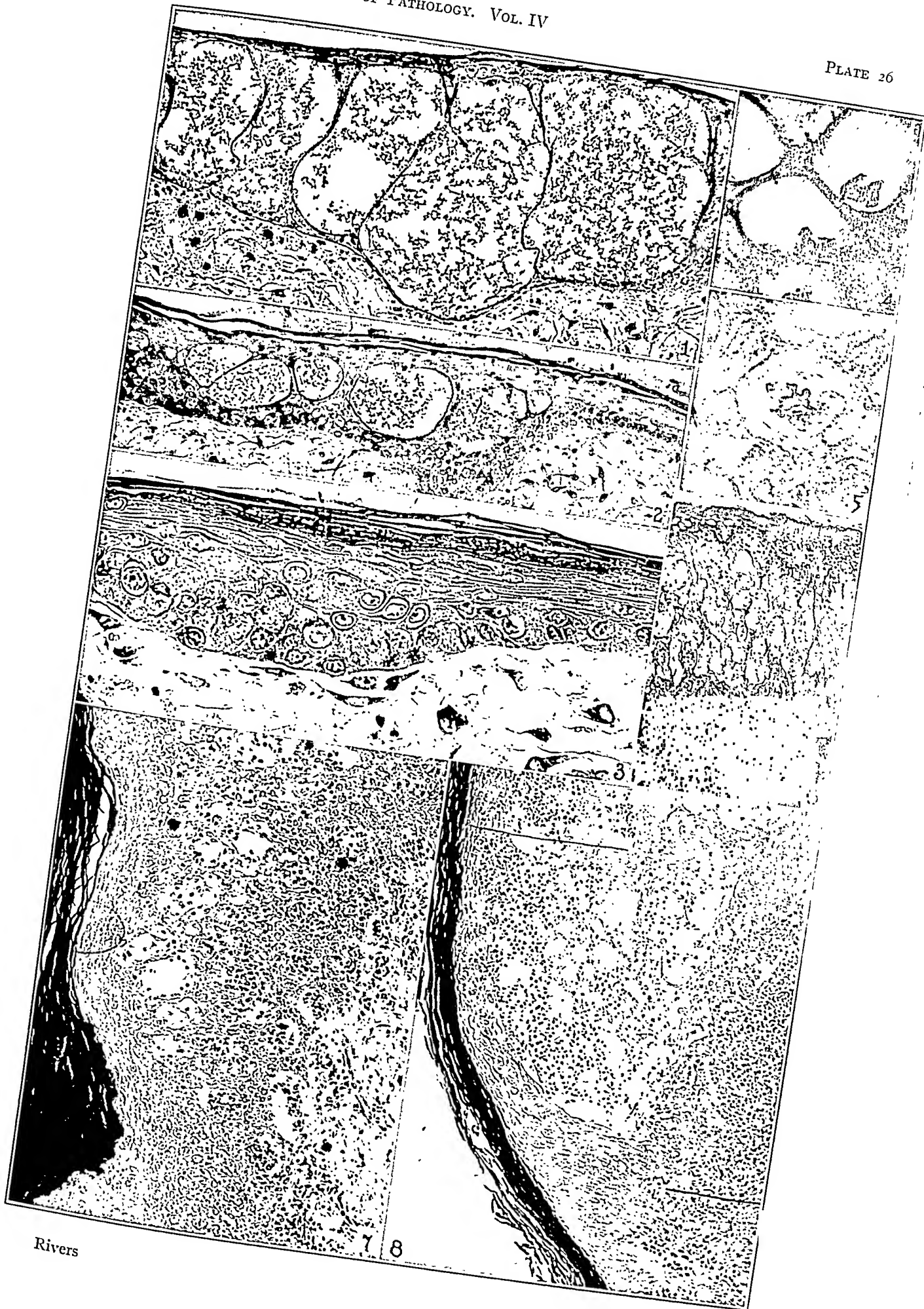


PLATE 26

- FIG. 1. Epidermal cells covering a myxomatous mass in the corium (virus myxomatosum, Sanarelli). Some of the epithelial cells are swollen and contain cytoplasmic inclusions. $\times 375$.
- FIG. 2. Epidermal cells undergoing dissolution. Compare with Fig. 1. $\times 125$.
- FIG. 3. Complete dissolution of epidermal cells. Compare with Figs. 1 and 2. $\times 125$.
- FIG. 4. "Degenerated cells showing vacuolation of protoplasm and shrivelling of nuclei." (After Councilman, Magrath, and Brinckerhoff, 1904.)
- FIG. 5. "Reticular degeneration of epithelial cell." (After Councilman, Magrath, and Brinckerhoff.)
- FIG. 6. "Early vesicle showing formation of epithelial reticulum." (After Councilman, Magrath, and Brinckerhoff.)
- FIG. 7. Vesicle in pad of guinea pig 18 hours after inoculation with virus of vesicular stomatitis. Reticulum formed by degenerated cells. $\times 125$.
- FIG. 8. Vesicle formation that frequently occurs in contagious epithelioma or fowl-pox. $\times 125$.



Rivers

Filterable Viruses

PLATE 27

- FIG. 1. "Crown tissue from rosette-diseased plant" (wheat). "Note the cells containing intracellular bodies in addition to the nuclei and also the granular nature of the cells which have become necrotic." (After McKinney, Eckerson, and Webb, *J. Agric. Res.*, 1923, xxvi, 605.)
- FIG. 2. "Crown tissue from healthy plant." Compare with Fig. 1. (After McKinney, Eckerson, and Webb.)
- FIG. 3. "Section of an early stage of a fruit blister showing the brown necrosis of the epidermal cells and the hyperplasia of the subepidermal cells." (After Gardner, *J. Agric. Res.*, 1925, xxx, 871.)
- FIG. 4. "Section of a pericarp lesion resulting from necrosis and collapse of an area of subepidermal cells and hypertrophy of the cells immediately beneath the necrotic area." (After Gardner.)
- FIG. 5. "Surface blisters on a mosaic tomato." (After Gardner.)
- FIG. 6. Mosaic leaf of a gourd. (After Doolittle, *U. S. Dept. Agric. Bull.*, No. 879, 1920.)
- FIG. 7. "Mosaic fruit of pumpkin, showing large dark-green swellings on a yellow background." (After Doolittle.)
- FIG. 8. Mosaic disease of Summer Crookneck squash. (After Doolittle.)

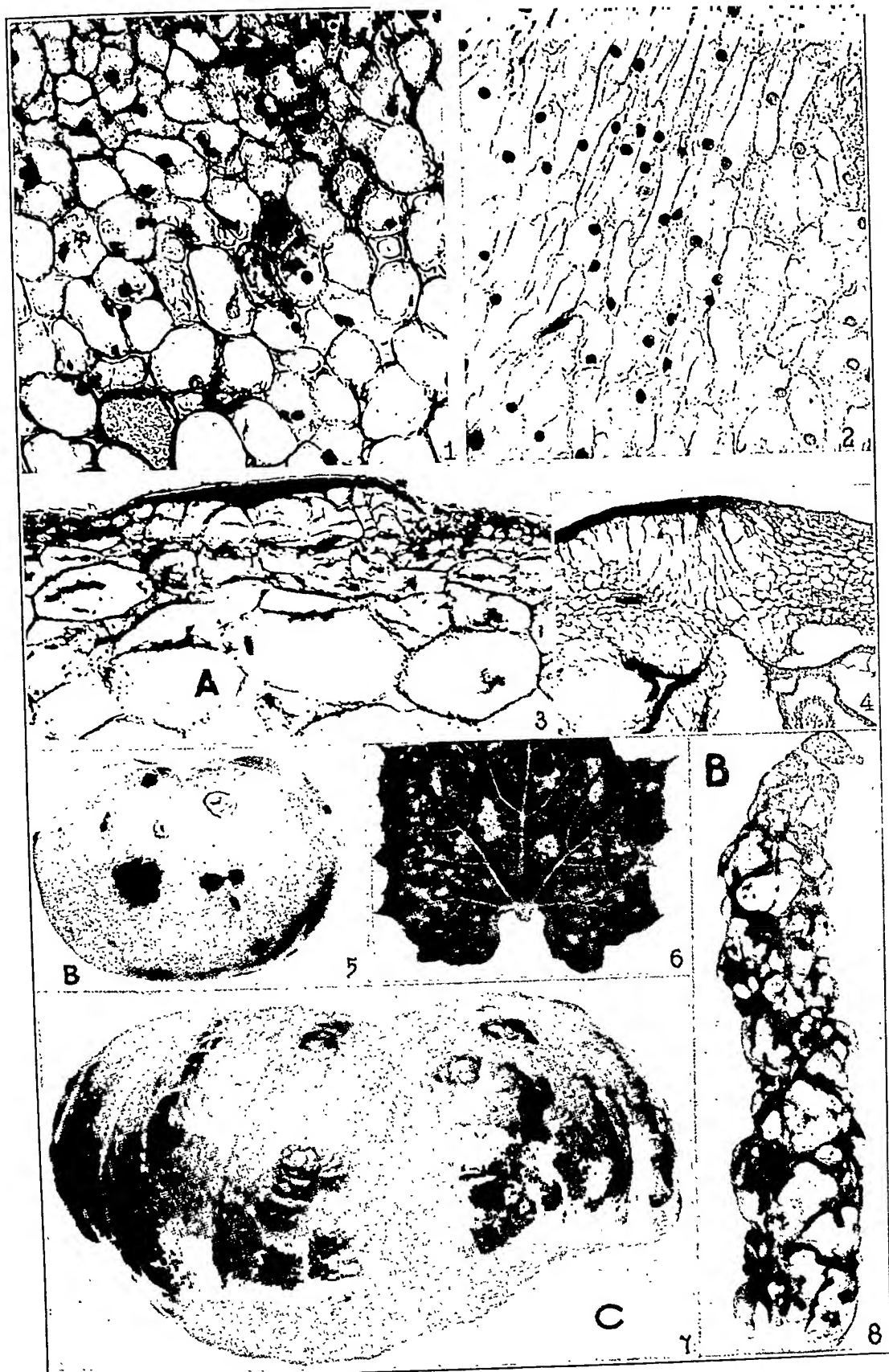


PLATE 28

FIGS. 1 and 3. Fig. 3 is an enlargement of Fig. 1. At the left side of Fig. 3 are normal bacteria. The large organisms in the center of the picture show the swelling induced by the action of bacteriophage. Pictures supplied by J. J. Bronfenbrenner.

FIG. 2. Stained preparation of bacteria undergoing lysis. The protoplasm is beaded and segmented. Picture supplied by J. J. Bronfenbrenner.



A STUDY OF THE HISTOPATHOLOGY OF THE SO-CALLED ADENOSARCOMA OF SWINE *

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To the student of comparative pathology, the occasionally encountered neoplasm of the kidney and sublumbar region of swine offers an interesting field of investigation. This tumor has been called by a great variety of names: adenoma sarcomatode, adeno-myosarcoma, sarcocarcinoma, sarco-adenoma, rhabdomyo-adenosarcoma and adenosarcoma, the last being the term most commonly used by pathologists in veterinary medicine and by meat inspectors. The variety of terms used to designate this tumor attests to the lack of agreement as to its true nature; however, little has been contributed to the literature of comparative pathology during the last two decades that might assist in clarifying a conception of this interesting neoplasm.

Kinsley¹ gives a brief description of the growth but fails to justify the use of the term adenosarcoma, while Day,² who was the first to describe these tumors of swine, gives an excellent description of the tumor and also presents a formidable array of evidence to substantiate his use of the term adenosarcoma. Most of the standard textbooks on pathology and veterinary medicine fail even to mention this growth, which suggests its comparative rarity, as well as the lack of information concerning it. After reviewing Kinsley's and Day's descriptions of this neoplasm it is evident that they use the term adenosarcoma because of certain resemblances which they believe it has to the embryonal adenosarcoma of the child's kidney. In fact Day embraces Herzog and Lewis'³ view explanatory of the histogenesis of the embryonal adenosarcoma of the child's kidney as probable in the case of such tumors in swine.

With such a paucity of literature it is not surprising that the term adenosarcoma has been taken for granted in referring indiscriminately to all tumors encountered in the kidney of swine. I believe that this designation places undue emphasis on the connective

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tissue constituents with a consequent disregard of the importance of the epithelial elements which commonly predominate.

REPORT OF CASES

During five years I have collected eleven specimens of the tumor under consideration. All of these were received from packing houses where clinical observations were necessarily limited and as a consequence the following data are somewhat meager.

CASE 1. The animal was slaughtered for food and subjected to the usual necropsy examination. One of the kidneys was involved in a large tumorous process. The entire mass was received at my laboratory.

The tumor measured 15 by 25 cm. It had an irregularly oval shape and was gray in color and firm in texture. The surface was smooth and covered by a white tough capsule. Fragments of what appeared to be renal tissue were scattered throughout part of the tumor near its attachment. The mass weighed 2200 gm.

CASE 2. A tumorous mass was observed in the body of the right kidney of a two-year-old Duroc sow which had been slaughtered for food. The carcass was otherwise in good condition.

The tumor was a firm, sharply circumscribed mass embedded in the body of the kidney, particularly involving the medullary portion. The growth approached the capsule at the hilum. It measured 4 by 7 cm., and was gray in color and somewhat irregular in shape. The surface was roughened and invested with a capsule and a freshly cut surface showed distinct mottling of the tissue.

CASE 3. A two-year-old Poland China hog was slaughtered for food and at necropsy a tumor was discovered in the sublumbar region adjoining and posterior to the kidneys. The general physical condition of the carcass was good; and no other structures were involved.

The mass was spherical and about 25 cm. in diameter. It was flesh-colored and soft. The surface was smooth and covered by a capsule. A cross-section of the mass revealed some irregular spongy areas, as large as 1 cm. in diameter, which contained light brown papillary projections. The tumor seemed quite vascular at the time of its removal from the carcass.

CASE 4. The animal was an eight-month-old Duroc male hog, which was slaughtered for food. At necropsy the carcass was found to be in good condition with the exception of a mass lateral and distal to the left kidney.

The tumor was a firm, bean-shaped mass, measuring 38 by 30 cm. and weighing nearly 8 kg. (16.5 pounds). The tumor was gray, rather vascular and the surface was covered with a capsule which could be removed without difficulty. It was also covered with a serous membrane.

CASE 5. A year-old male Duroc hog was slaughtered for food and the carcass subjected to the usual necropsy examination. A diffusely attached tumor was observed involving the region of the hilum of the left kidney.

The tumor had an irregular roughened surface and measured about 5 by 5 cm. It was firm in consistence and of a grayish white color with a few dirty yellow areas scattered throughout its substance.

CASE 6. A hog in apparent health was slaughtered for food. During the usual necropsy inspection of the carcass a mass was observed near the surface at the end of one kidney.

The tumor was a grayish oblong structure with a slightly roughened surface surrounded by a very thin capsule. The mass was firm in some places and spongy in others. It measured 12.5 by 6 by 4.5 cm.

CASE 7. A hog was slaughtered for food and a neoplasm was found in the position ordinarily occupied by one of the kidneys although but little renal tissue was present.

The mass measured 20 by 10.5 by 20 cm., was gray, quite firm, and was surrounded by a capsule. It was oval and flattened. A freshly cut surface showed strands of grayish white tissue cutting the tumor into small irregular lobular areas.

CASE 8. A hog had been slaughtered for food after having passed a satisfactory antemortem inspection. When the carcass was eviscerated one kidney was found to possess a number of grayish white areas of varying sizes, most of them slightly raised above the surface (Fig. 1).

The kidney was cut through longitudinally and the grayish tissue was seen in some places extending almost through the cortical portion of the organ. Freshly cut sections of the kidney through the grayish white areas showed them to be diffusely in contact with the substance of the organ, with no attempt at encapsulation. The line of contact of the tumorous tissue with that of the kidney was quite uneven. The tumor was of about the same consistence as the kidney proper.

CASE 9. At antemortem examination there was no manifestation of the tumor which occupied the left sublumbar region of a two-year-old female hog which had been slaughtered for food. The tumor was rather massive and measured 42 by 28 cm. in its greatest dimensions. It weighed 9.5 kg. (about 19 pounds). The renal tissue had practically disappeared and all that remained of the organ was a flattened atrophic layer on the surface at one side of the tumor. The mass was elongated and quite irregular (Fig. 2). When the tumor was cut through, multiple cysts were found which contained foul-smelling fluid. The interior of the tumor was mottled. Cartilage or bone was not demonstrable.

CASE 10. A hog, age and sex not given, was slaughtered for food and a tumor of the left kidney was observed.

The kidney showed a roughened nodular protuberance at one pole which was of a lighter color than the rest of the organ. When the kidney was divided longitudinally it was found that practically the entire interior consisted of abnormal tissue with the renal substance limited to a shell-like structure at the periphery. The tumor was attached firmly in the region of the protuberance while the bulk of the mass was invested with a capsular covering which seemed to be deflected to line the renal cavity in which the tumor rested.

CASE 11. A seven-month-old hog was slaughtered for food. The animal was in fair condition and there was no antemortem evidence of disease. Both kidneys were affected with diffuse lobulated tumors. The right kidney measured 15 by 9.5 cm. and weighed 540 gm. The left kidney measured 20 by 10 cm. and weighed 810 gm. Tumors presented a compact, mottled type of structure with but little kidney tissue remaining (Fig. 3).

GROSS CHARACTERISTICS

Site: These neoplasms may involve the renal tissue, or, in extreme cases in which the renal tissue has been replaced by the tumorous proliferation, they are found in the place ordinarily occupied by this organ and extend backward into the pelvis and forward perhaps to the diaphragm. Occasionally the kidneys are intact and the tumor appears posterior to the kidney in the sublumbar region. The tumors are usually unilateral but bilateral involvement may be encountered (Case 11).

Shape: The shape is variable, the growths for the most part being oval but often presenting an irregular contour with deep lobulations.

Size: In bulk the tumors vary from a small focus just visible to the eye to huge masses weighing several kilograms. Day reports one that weighed nearly 27 kg. and another that weighed 12 kg. The largest in my series measured 42 by 28 cm. and weighed 9.5 kg. (19 pounds). In a personal communication received recently from Dr. E. W. Barthold, Austin, Minnesota, he mentioned that several years ago he had encountered a specimen which weighed more than 31.5 kg. (66 pounds). This must approach the maximal size for these tumors.

Color and consistence: The majority of these tumors are grayish white, and they are rather firm. Some show dirty yellow areas scattered promiscuously throughout; those that are soft often are flesh-colored.

Capsule: In the larger specimens a capsular covering is always present which is never particularly adherent and is not difficult to remove. The extremely large tumors may have a serous covering which is a deflection of the peritoneal membrane carried along with the tumor as it grows and encroaches on the abdominal space.

Retrogressive changes: Cystic cavities are frequently a feature of the larger growths. The contents of the spaces may be serous fluid of a gelatinous nature. Occasionally spongy areas of variable dimensions are seen in the depths of the tumor. These are usually darker than the surrounding tissue and probably represent phases of necrosis. In the larger specimens there is sometimes solution of tissue with resultant foul-smelling purulent liquid occupying the necrosed areas. Freshly cut surfaces frequently present a distinctly mottled

appearance due to strands of grayish white fibrous tissue which cut the tumor into small irregular lobular areas (Fig. 3).

PATHOLOGIC HISTOLOGY

A microscopic study of the eleven tumors shows that they fall into two rather distinct groups. Seven tumors (Cases 1, 2, 6, 7, 8, 9, 10) are purely epithelial in nature with the connective tissue elements serving as nothing more than stroma for the epithelium of the parenchyma, without the slightest indication of malignant behavior. The other four tumors (Cases 3, 4, 5 and 11) should be considered as mixed tumors with a vigorously growing epithelial portion making up the bulk of the growths and minor areas of embryonic fibroblastic tissue exhibiting certain characteristics which simulate those of fibrosarcoma. To the second group the term embryonal adenosarcoma might be properly applied, while those first described would be correctly considered embryonal adenocarcinoma. Briefly, the morphologic features of the two groups follow:

Adenocarcinoma: With few exceptions the make-up of this type of growth is quite cellular, the parenchyma being irregularly interrupted by broad septums of connective tissue which often carry a few blood vessels. From these larger septums smaller strands are frequently given off which pass into the epithelial areas to become lost in the complex maze of their ramifications. The type of cell may be either the low cuboidal to low columnar variety or a peculiar elongated spindle-like cell which takes an intense nuclear stain. The cytoplasm of the latter variety may be slightly granular and the nucleus, which resembles an oat kernel in contour, usually occupies a considerable portion of the cellular bulk. Owing to the intense basic-staining qualities of the nucleus it is usually impossible to study anything more than the contour and size of this cell. Occasionally a tumor is encountered in which both types of epithelial cell are represented, some portions showing the low columnar or cuboidal variety, while in other areas the spindle type of cell with the deeply staining nucleus, shaped like an oat kernel, constitutes the parenchyma (Case 9, Figs. 4 and 6).

In areas in which the spindle type of cell is present one often finds a peculiar tuft-like arrangement of the parenchyma, which suggests a renal corpuscle (Figs. 4 and 5). Capillary vessels are absent, the

structure consisting only of convolutions of flattened epithelium and fibrous stroma within a cavity which is lined with a continuation of the small flattened cells that constitute the tuft-like portion. Usually the cells of the parenchyma are arranged in an irregular tubular or alveolar fashion with occasional acini and epithelial papillae projecting into the cavities (Fig. 6). In many instances the epithelial cells are not confined to the lining of the tubular or alveolar spaces but in an undifferentiated state are promiscuously disposed in great numbers in the intertubular areas. Mitotic division of the epithelial cells is often seen and eosinophils are occasionally observed in the intertubular or interalveolar areas. In the larger specimens of this tumor, the renal tissue may be entirely replaced by the neoplastic elements, while in the earlier types the tumor cells may be seen invading the surrounding renal substance in a most menacing fashion. This type of tumor is similar in most details to the characteristic adenocarcinoma found elsewhere, except perhaps in the matter of metastasis.

Adenosarcoma: A phenomenon in the growth of adenosarcoma is that the fibroblastic elements in many of the areas ordinarily looked on as stroma exhibit the same malignant neoplastic tendencies as the adenomatous portions described for adenocarcinoma. In certain fields these fibroblastic cells have a distinct embryonic appearance and mitosis can be frequently demonstrated. The epithelial portions are distinctly adenocarcinomatous (Figs. 7 and 8).

In the mixed type of tumor it is not unusual to find epithelial duct-like structures in the midst of fields of young proliferating fibroblasts. With Van Gieson's stain one occasionally finds, in certain portions of the stroma, some strands of tissue with the characteristic light yellow color of smooth muscle. Day and others have described the occurrence of cartilage and bone in certain portions of these tumors but I failed to demonstrate either of these tissues in the material at my disposal.

In *both types* of the tumor, cavities or cysts of variable size may be encountered. Some are microscopic while others measure from one to several centimeters in diameter. These are usually lined with a single layer of cuboidal epithelial cells. The content of these cavities is a homogeneous substance which stains pink with eosin and resembles, in most respects, epithelial hyalin although the color reaction with Van Gieson's stain is not entirely typical for

this material. Areas of hydropic degeneration associated with necrosis of the tumor cells are occasionally encountered. Ample blood supply is insured by the many vessels running in the stroma of connective tissue and by the capillary structures which are sometimes seen in the midst of the cellular parenchyma.

Metastasis: While *both types* of the tumor are capable of exhibiting a destructive, advancing type of growth in so far as the renal substance is concerned, metastasis to the regional lymph nodes and to distal parts of the body, while possible, is seldom observed. Day described metastasis in two cases, the lung being involved in one and the sublumbar lymph nodes in the other.

In none of my cases was metastasis demonstrable, although it must be admitted that a thorough search for secondary foci was not made at the time the carcasses were eviscerated because of the lack of time and the failure of the examiner to appreciate the importance of this phase of the necropsy.

Wollstein⁴ contends that metastasis from these tumors in the child is hematogeneous and that the regional lymph nodes are seldom invaded. Packard and Blumberg⁵ report that in the child metastasis may appear in the vertebrae. This observation in swine has not been reported.

SYMPTOMS

Compared to the closely related neoplasms of man which are considered to be particularly malignant, the variety in swine seems benign. In man they produce symptoms of a grave organic disturbance and usually terminate fatally in the first few years of life. In children metastasis to lung or liver is common. In swine, on the other hand, there has been no instance, so far as I can determine, in which these tumors have been diagnosed or even suspected during life. That these tumors in swine are not detected during life may be accounted for at least in part by the horizontal posture of this animal. This position causes the abdomen to be more or less pendulous and as a result large renal tumors may press downward unnoticed in a part of the body which is normally of imposing dimensions. In the child the upright posture and the anatomic configuration of the abdomen render the detection of such tumors fairly easy even before they attain large proportions. Even in those animals in which, at necropsy, large tumors were found, symptoms of suffi-

cient magnitude to command attention were absent. These large tumors must interfere to some extent with the digestive process by extension into the space ordinarily necessary for the proper housing of the abdominal viscera. No doubt the animal experiences sensations of discomfort from large abdominal neoplasms, such as man experiences. If the tumor is unilateral and involves the kidney proper there must be gradual compensatory hypertrophy of the unaffected organ. In the rare cases of bilateral renal involvement, grave consequences will certainly follow the gradual encroachment of the neoplasm on the renal substance.

The fact that most swine are slaughtered at a comparatively early age, undoubtedly accounts for the failure to observe the symptoms which might be expected from tumors of such menacing possibilities. Perhaps the tendency of the tumor to become encapsulated assists in preventing its spread. If metastasis occurs and the disease is permitted to run its course, the usual effects of malignant disease can be predicted.

HISTOGENESIS

In studying these tumors one cannot avoid the conclusion that they bear a close relationship to the embryonal renal tumors occasionally observed in children, usually referred to as embryonal adenosarcoma, although there is not complete agreement as to the use of this term. The histogenesis of these growths offers an interesting field of speculation and many theories have been offered in explanation. While the hypotheses of various authors differ in many respects, there seems to be agreement that these tumors develop as a result of some congenital mishap. The exact nature of the anomaly is not yet determined, although there is suggestive evidence that it is the result of some fetal inclusion, a fetal rest or a fetal displacement.

Wollstein in a recent paper presents a logical explanation of the origin of these tumors. She states: "As to the origin of these renal neoplasms it seems probable that they all come from the same type of embryonal cell, but that they come from the cell at different stages of its differentiation and consequently of its potency. The more embryonic and less differentiated the cells from which the tumor originates, the more complicated will its structure be because of the multipotency of the undifferentiated mesodermal cells. Thus

for the rhabdomyo-adenosarcomas it seems simple to presuppose a parent cell with an inherent ability to give rise to striated muscle, supporting tissues and kidney elements, since these are all mesodermal in origin and closely approximated in the young embryo, and the inclusion of such early cells within the developing kidney is possible. This was Wilms'⁶ view. It seems the only way to account for the striated muscle tumors, for striated muscle cannot develop from unstriated muscle, and so metaplasia gives no adequate explanation of these growths. The presence of cartilage and bone in the mixed tumors is also explained. For the less complex tumors two possible origins suggest themselves. Either the parent cells are more differentiated, of later origin, and so have the potentiality only of the renal and supporting tissue elements or the cells of the early renal blastema are adequate for the development of the tumors containing only kidney elements like tubules, connective tissue and unstriated muscle. Again, growths with more embryonal epithelial elements may take their origin from an earlier cell of the renal blastema than that which gives rise to the more adenomatous forms, when the cells have reached the stage of differentiation of renal tubular epithelium. Since these neoplasms are not all alike, but only closely related, there seems no special reason why a single origin must be involved for them as a group."

This is, in the main, the opinion of Nicholson⁷ who believed it "reasonable to conclude that such tumors have originated in areas of undifferentiated kidney substance which are known to occur." Fraser⁸ attributed the origin of the adenomatous tissue to a group of erratic nephrogenetic cells and of the sarcomatous tissue to metaplasia of the adenomatous elements. Neither of these authors believed that the Wolffian body was responsible in any way for the origin of the growths.

Certainly it is not necessary to use the Wolffian body to explain tumors of this type which occur, as the majority do, within the substance of the kidney proper. An occasional tumor of this variety is found entirely separate from the kidney and it would seem logical to assume that the Wolffian body was at least indirectly implicated in its origin and that the anomaly probably results from failure of certain portions of the mesonephros to be utilized in the production of permanent renal tissue. This failure results in so-called fetal rests from which the tumor develops. Considering the early age at

which these tumors become manifest, it is hardly likely that the forerunner of tumor tissue remains long inert. On the contrary, there probably never is cessation of growth. It proceeds without the directing influences of normally developing tissues and becomes an erratic, ungoverned mass of cells with all the characteristics and possibilities of true tumor.

The occurrence of the embryonic neoplasms apart from the kidney must be rare since I have found only two cases (in man) in the literature reviewed. Hedren's case quoted by Wollstein presented bilateral involvement in which the kidneys, while compressed by the adjacent tumors, were intact and not invaded by neoplastic cells. Hood and Albert⁹ also described an embryonal tumor in a child whose kidney was free from neoplastic involvement although flattened by the proximity of the growth. This case was interesting in that evidence of slight metastasis was observed in the adjacent intestinal loops. The kidney in contact with the tumor was described as showing marked fetal lobulation and slight hydronephrosis. In two of my cases (Cases 3 and 4) the tumor was in the sublumbar region and did not involve the kidney. Both were of the mixed type and could be correctly designated as embryonal adenosarcoma. The occasional observation of these tumors posterior to the kidney suggests the possibility that they occur anterior to this organ as well. The tuft-like arrangement of the flattened cells in certain of the adenocarcinomas of this group (Fig. 4) suggests further that these tumors arise from nephrogenetic cells at different stages of differentiation. This structure has a general resemblance to a renal corpuscle (Fig. 5). On close examination the cells in this arrangement appear similar in many respects to the flattened epithelial cells constituting Bowman's capsule of the normal kidney.

It would appear that cells, which were destined in the ordinary course of events, to invest the glomerulus, had undergone some differentiation in preparation for their normal purpose, then had suddenly lost the directing influence of normal nephrogenic tissue, and as a consequence of this loss of restraint had continued to proliferate in a haphazard, aimless manner. Occasionally they are found lining narrow crescent-shaped cavities with the inner layer arranged in folds or convolutions suggesting that the true function of the cells is to line the glomerular space and cover the glomerular structure. However, since glomerular elements are missing, the cells intended

to invest them proliferate and practically fill the cavity with multiple folds of flattened epithelial cells.

DISCUSSION

The occurrence of tumors of this type in swine further emphasizes the fact that neoplasms fail to respect species. Practically every kind of tumor which may affect man has been observed in the lower animals. It is a little peculiar, however, that these embryonal growths should be limited among the lower mammals to swine, for there is no evidence that they have been observed in other species. Why swine alone should be susceptible to these tumors and other mammals escape offers an interesting problem for the embryologist.*

The early age at which the majority of these tumors are found, especially in children, suggests a congenital inception. When these renal tumors occur, children are far from the so-called cancer age, indicating that the growth, perhaps in many cases, is well under way at birth. In this connection it is interesting to note that Kastner, quoted by Wollstein, found a spindle-cell sarcoma of the kidney in a seven-months fetus. Nicholson mentioned a case of adenosarcoma of the left kidney in a woman aged forty. In this instance growth must have been very slow which is in opposition to the behavior of the majority of these tumors in man.

The life of swine compared to that of man is short and as a consequence it is difficult to determine whether there is an age in swine when tumors are more prone to occur. Available data would indicate that the tumors in question are the most commonly encountered neoplasms in swine. That these tumors are seldom observed suggests further the relative immunity of swine to neoplasms which arise except as a result of congenital anomaly. In a previous communication¹⁰ dealing with the incidence of tumors in the lower animals, it was shown that of a total of 132 tumors which were collected for study in a period of more than four years, nine species were repre-

* Since this article was written I have reviewed the papers of Bell and Henrici,¹² Scott¹³ and Polson¹⁴ on embryonal tumors of the kidney of the rabbit. Also that of Baird¹⁵ who described an embryonal epithelial tumor from the right kidney of a chicken which he concluded was the "remnant of an early stage renal anlage." Through the courtesy of Dr. Frank P. Mathews, La Fayette, Indiana, I have also had the privilege of studying several tumors of the kidney of the chicken; these are unquestionably embryonic in nature and similar in most respects to those of the kidneys of swine.

sented. Fifteen of these tumors occurred in swine and of these, nine (60 per cent) were tumors of an embryonal nephrogenic type. The other six were; myxoma one, melanoblastoma three, lymphosarcoma two. While this is perhaps a rather small series from which to draw conclusions, the data at least suggest the relative freedom of swine from the ordinary epithelioblastomas which are so common in the cow and horse.

As to the nomenclature of these newgrowths, it has been the custom of most observers to consider all of them, both clinically and histologically, as adenosarcoma. I prefer the term embryonal nephroma. So far as gross characteristics go there can be no special objection to either of these terms which have become fixed by long usage, but microscopically it is possible and desirable to separate these tumors into two and possibly three groups, although for the cases described in this paper two groups would suffice.

While the groups unquestionably have many features in common, I fail to see any good reason why all should be labeled the same histologically. There is justification for the use of a common term by which they may be designated grossly since structural detail cannot be determined macroscopically, but there is no reason why the pathologist should use a single term since differences in structure are apparent to anyone who has the opportunity to study these growths histologically. While in every case the microscopic picture is embryonal in character, there is often considerable difference in the dominant element. This difference also obtains in the tumors of this type observed in children. Wollstein spoke of this feature in her recent paper and it was also mentioned by Hinman and Kutzman.¹¹

In tumors in which the epithelial elements constitute the major portion of the structure with the connective tissue elements clearly acting as supporting stroma, as in glandular epithelioma, it would seem proper to use the term embryonal adenocarcinoma. To designate these as adenosarcoma is without good reason, since cells with sarcomatous tendencies are lacking. Those with a mixed type of architecture, possessing in addition to epithelial nests and tubular structures a variable amount of embryonic connective tissue elements such as fibroblasts, bone, cartilage, smooth and striated muscle, could be designated embryonal adenosarcoma indicating the adenomatous structure associated with tissues possessing sarco-

matous tendencies. I have not observed an example of the third group in the relatively few specimens I have studied. Wollstein, however, described a typical fibrosarcoma from the kidney of a six-month-old male infant. The structure was devoid of any epithelial elements and the connective tissue stroma was scant. The growth recurred soon after operation and the disease terminated fatally three and a half months after the initial symptoms, which attests to the rapidity of growth possible in this type of embryonal neoplasm. While a pure embryonal fibrosarcoma from the kidney of swine has not been described it would be presuming too much to say that it does not occur. A proper examination of all tumors of the renal area would undoubtedly reveal this type which of course must constitute a minority of these peculiar tumors.

SUMMARY AND CONCLUSIONS

A review of the literature indicates that these tumors have received but scant attention from the standpoint of comparative pathology.

Eleven embryonal tumors of swine are described, nine of which had their origin in the kidney and two posterior to this organ in the sublumbar region.

A comparison of the specimens from swine with the embryonal renal tumors of the child indicates that the process is comparable in the two species and that sufficient similarity exists to justify the assumption that there is a common histogenesis.

The two tumors in the sublumbar region possibly owed their origin to the failure of certain portions of the mesonephros to be used in the development of the permanent kidney.

The embryonal renal tumors of swine, like those of the child, constitute a heterogeneous group. Grossly it is difficult if not impossible to separate them but histologically two, or perhaps three, types exist. They fall under the following headings: embryonal adenocarcinoma, embryonal adenosarcoma, and embryonal fibrosarcoma. A pure embryonal fibrosarcoma of the kidney of swine has not as yet been described.

Available data indicate that this group of tumors constitute the most common neoplasm of swine, a species which seems to possess a relative lack of susceptibility to tumorous proliferations.

Unlike the closely related group of tumors of the child, those of swine do not appear to embarrass the health. Perhaps this is due in part to the relatively early age at which most swine are slaughtered.

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DESCRIPTION OF PLATES

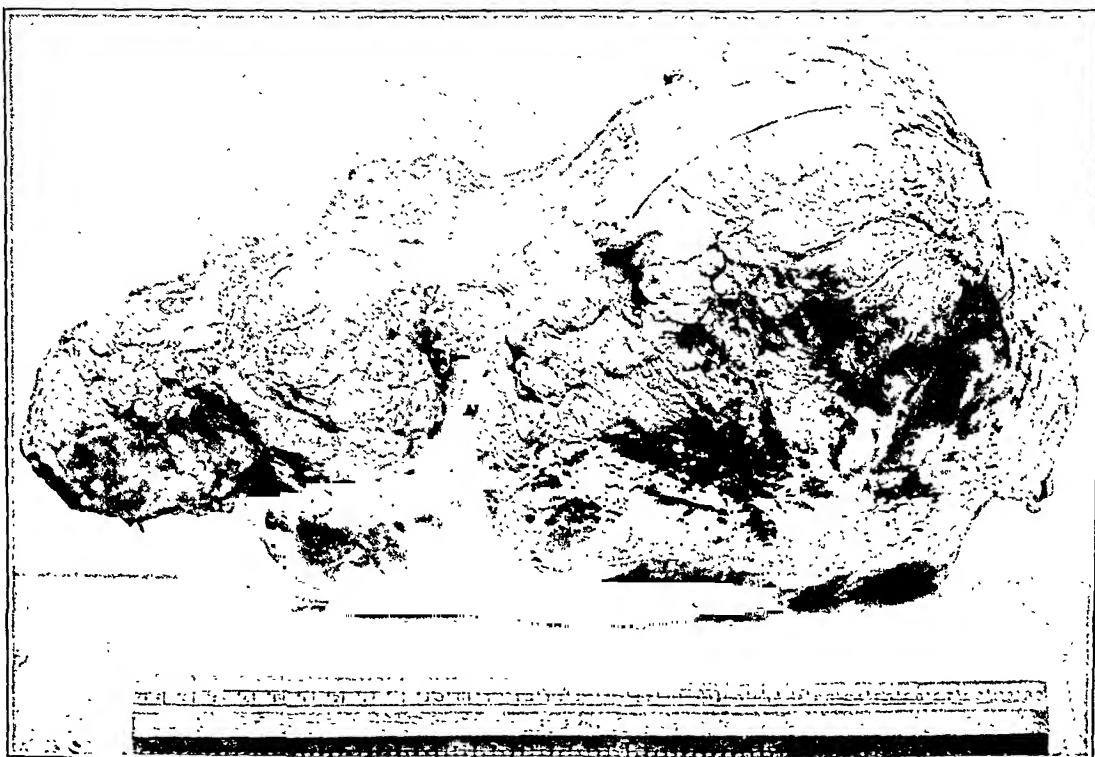
Plate 29

FIG. 1. (Case 8.) Extensive renal involvement by the tumor which presents multiple foci. The distribution of the tumorous tissue is clearly shown in the cortical zone and over the surface of the organ. This specimen represents an earlier stage of the disease than is usually seen.

FIG. 2. (Case 9.) Gross appearance of tumor. Remaining renal tissue so small in amount as to be practically indiscernible.



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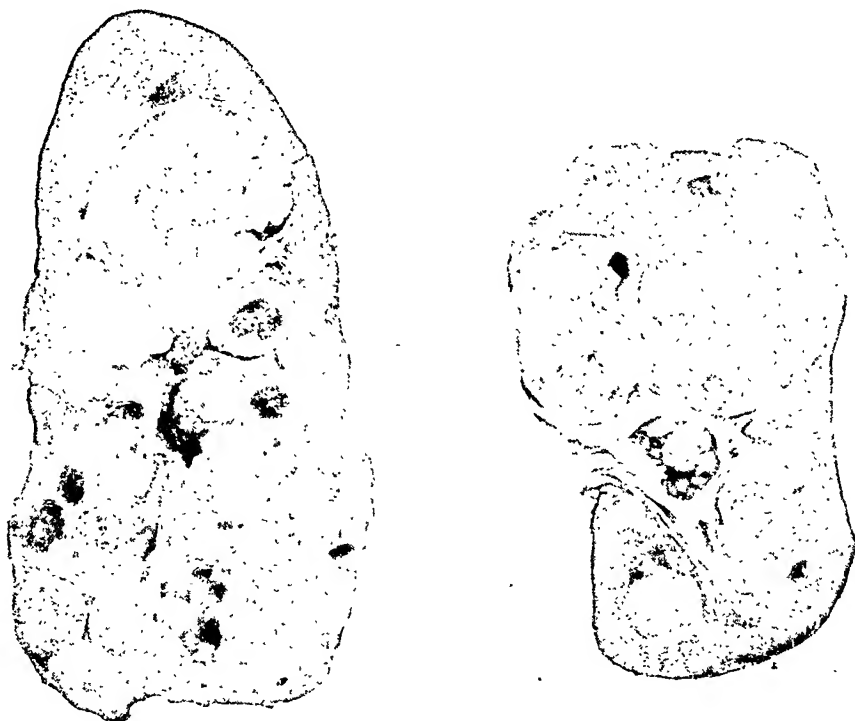


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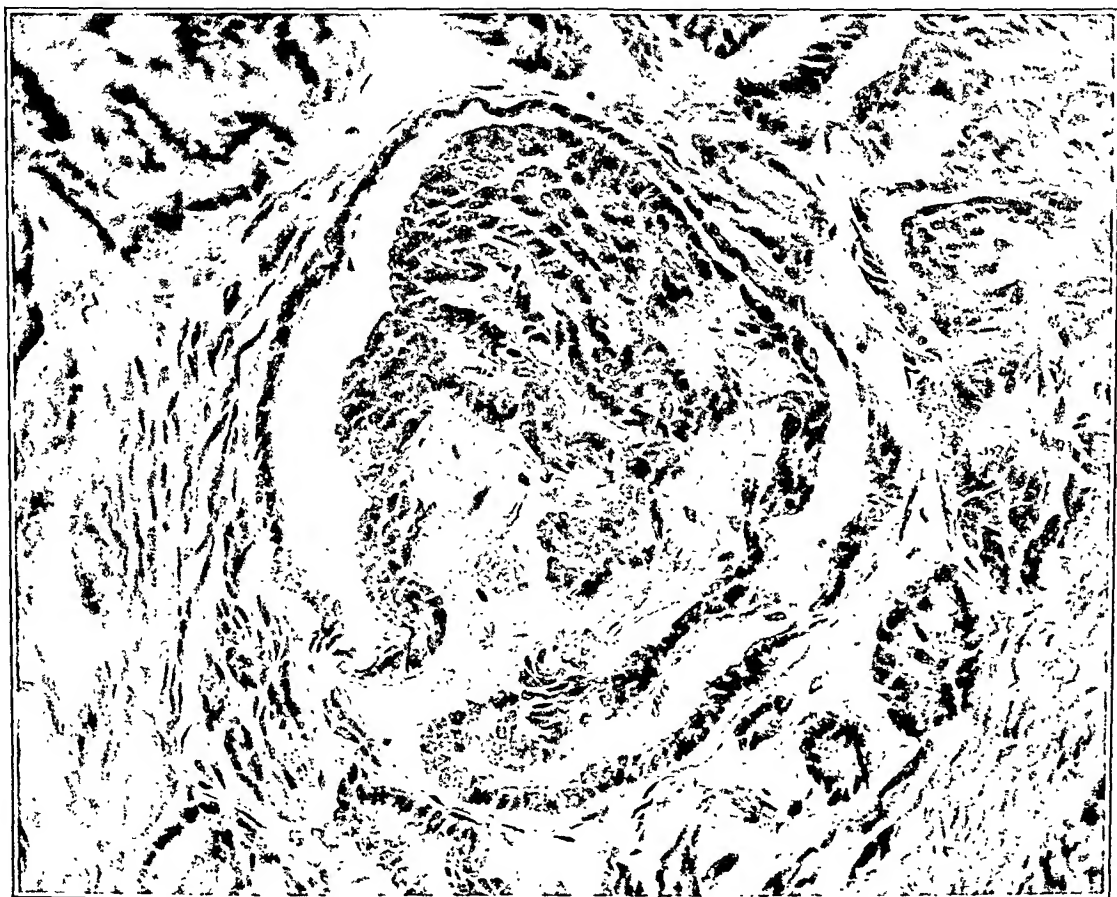
PLATE 30

FIG. 3. (Case 11.) Bilateral embryonal adenosarcoma. Longitudinal sections of left and right kidneys which show the small amount of kidney tissue present and the mottled lobular character of the tumors.

FIG. 4. (Case 9.) Tuft-like arrangement of the flattened epithelial cells. The architecture of this embryonal adenocarcinoma has some general resemblance to a renal corpuscle. Compare with Fig. 5. $\times 400$.



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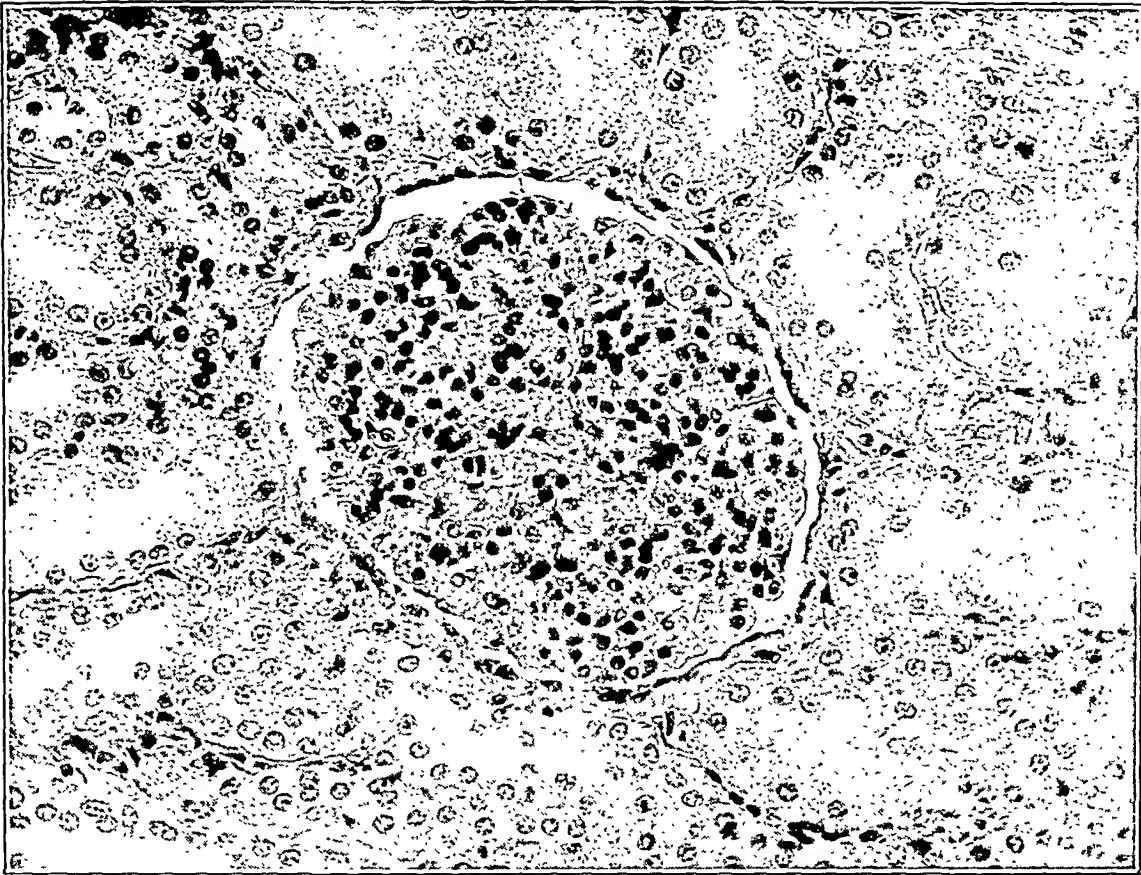


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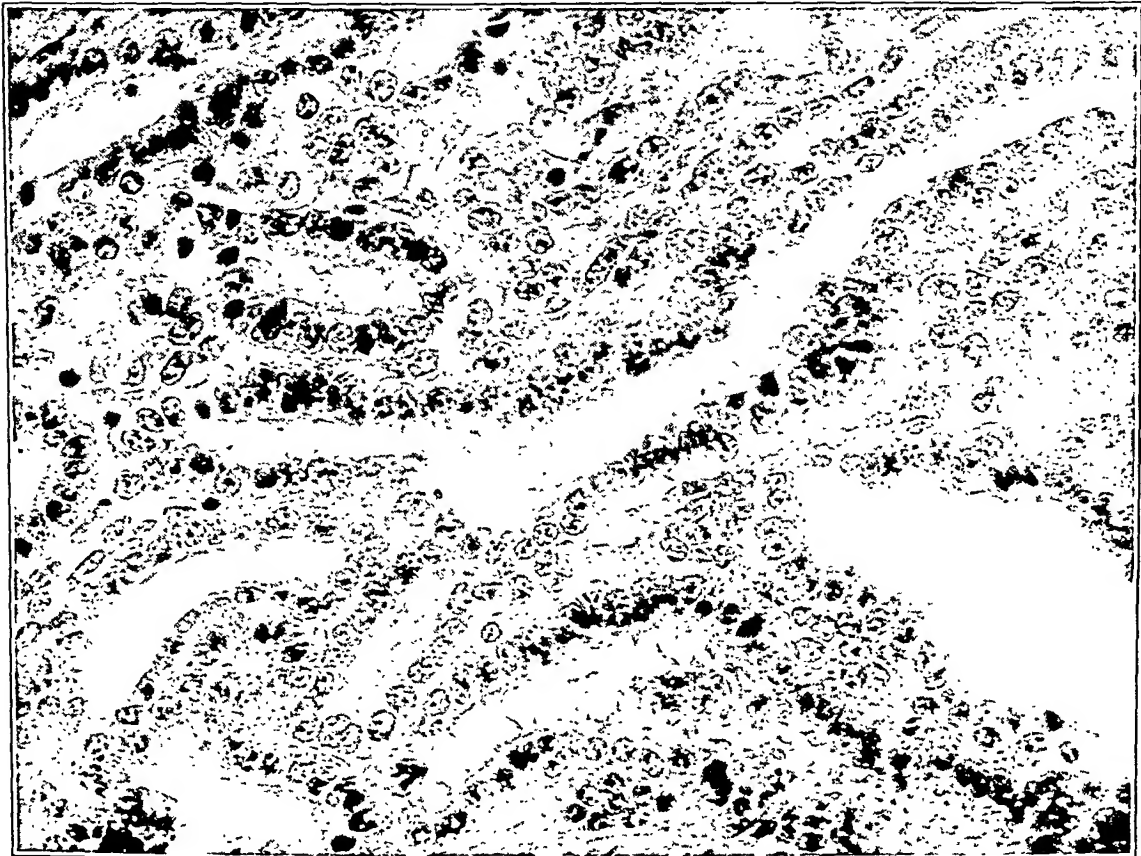
Plate 31

FIG. 5. A portion of a normal kidney of the swine showing one renal corpuscle.
× 400.

FIG. 6. (Case 9.) Highly cellular tubular type of architecture of an embryonal
adenocarcinoma. × 575.



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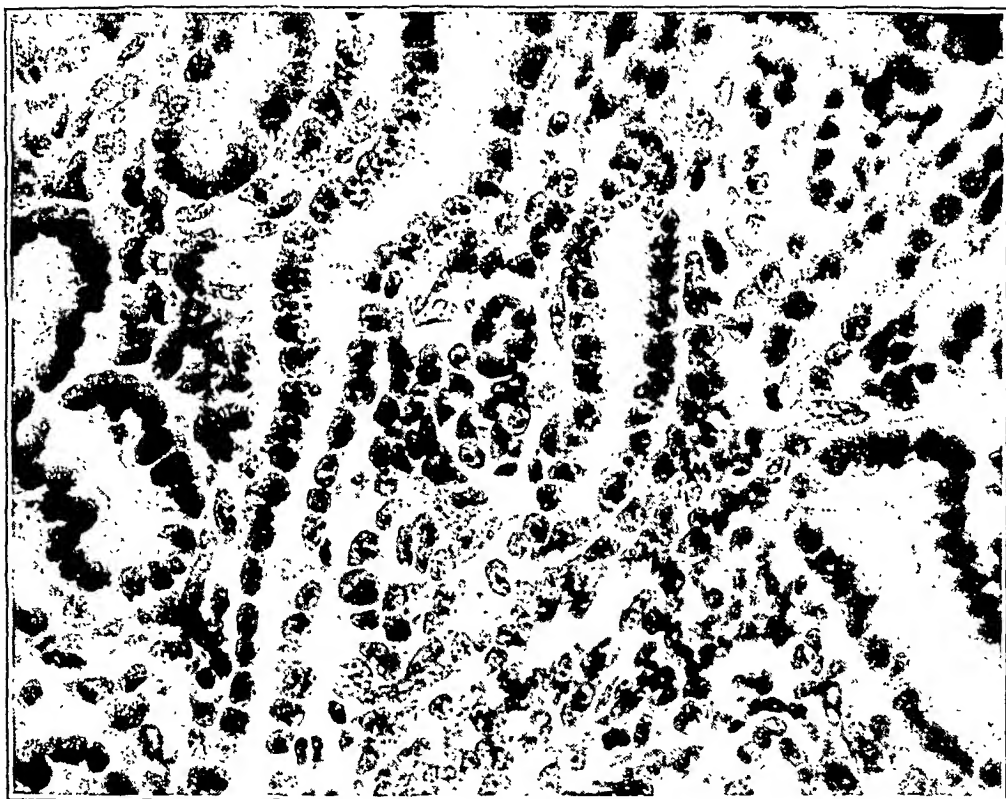


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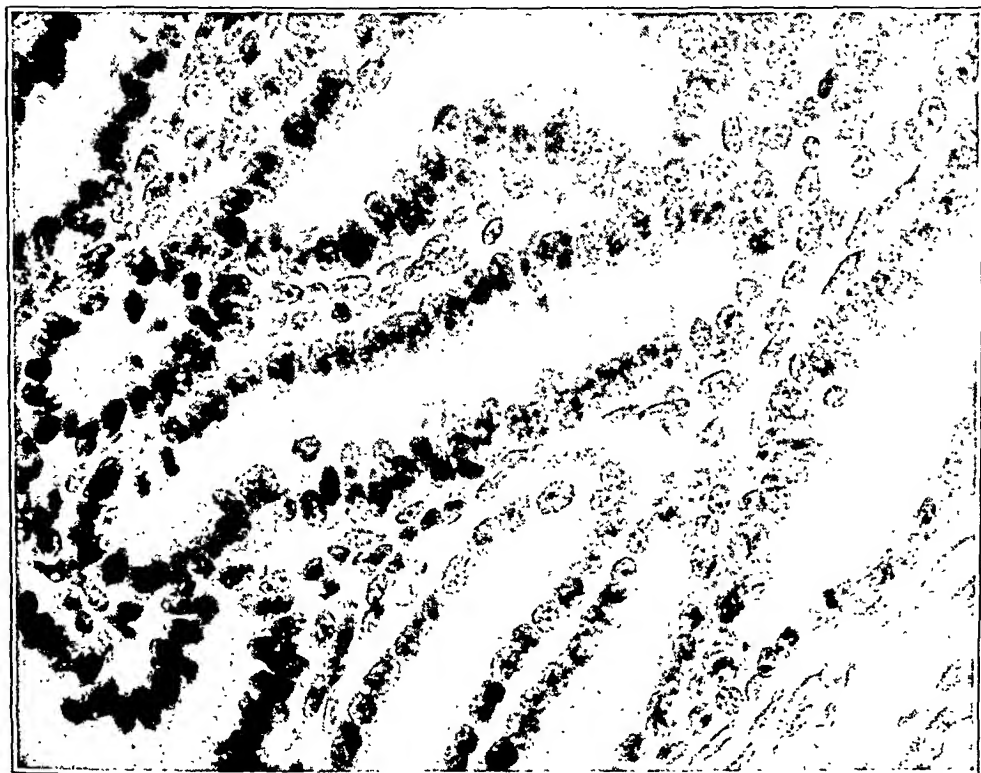
PLATE 32

FIG. 7. (Case 4.) The gradual transition of the epithelial cells lining the tubular-like structure from cuboidal to columnar is shown. An epithelial portion of an embryonal adenosarcoma. $\times 600$.

FIG. 8. (Case 5.) An epithelial area of an embryonal adenosarcoma showing the tubules lined by a single layer of cells several of which show mitotic division. $\times 600$.



7



8

LEIOMYOSARCOMA OF THE SPLEEN IN A BOVINE *

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The relative infrequency of splenic tumors in human beings has been commented on by many writers. Certainly there is little on the subject in the available literature. Smith and Rusk¹ state, "Malignant neoplasms arising primarily in the spleen are found so infrequently as to be considered pathologic curiosities and so have been of little interest to the clinician." In a recent comprehensive paper on splenic neoplasms by Krumbhaar² the following appears: "Splenic neoplasms are of especial value in the study of cancer as well as of the spleen on account of their comparative rarity and the relation that this may have to the supposed rôle of the spleen in combating tumors and infections." Krumbhaar reports 6500 necropsies which yielded a total of 930 primary and 1234 secondary tumors of which only forty occurred in the spleen. Of the forty splenic tumors six were primary and thirty-four were secondary. Leiomyoblastoma of the spleen was not observed. Smith and Rusk noted reports of 104 splenic tumors and reported two original cases which they considered endotheliomas. They did not observe a case of leiomyoblastoma, and considered lymphosarcoma the most common tumor of the spleen.

According to Smith and Rusk benign tumors of the spleen are not infrequently found both in man and in lower animals. If one excepts the so-called lymphomatous tumors of the dog's spleen which are fairly common, but which may not be true tumors, the statement of these writers relative to splenic tumors in animals is not substantiated by the literature or by my own observation. In fact literature on comparative pathology contains practically nothing pertaining to splenic neoplasms in lower animals. Hutyra and Marek³ mention only the melanosarcoma of the horse's spleen, and Fox⁴ failed to observe any splenic tumors in a series of ninety-four neoplasms from more than 5000 captive wild mammals and birds. In an observation of approximately 250 tumors representing nine dif-

* Received for publication January 9, 1928.

ferent species of the lower animals over a period of ten years I have encountered primary splenic tumor only once; this case is reported here. Secondary tumors of the spleen in dogs and chickens affected with lymphocytoma are not uncommon.

I have failed to note in the literature any case in man or animal in which leiomyoblastoma originated in the spleen. MacCallum ⁵ believes that the walls of the uterus are the usual site of origin of leiomyoblastoma, and that the kidney, bladder and walls of the stomach and intestines are rarely involved. He does not mention its occurrence in the spleen. Mallory ⁶ says that aside from lymphoblastoma other primary tumors of the spleen are rare. He also believes that the uterus is the most common site for the development of leiomyoblastoma, and that occasionally a tumor of this variety arises in the kidney and subcutaneous tissues. Its presence in the spleen is not considered. Ewing ⁷ states that primary sarcoma of the spleen is rare; he does not mention the occurrence of leiomyoblastoma in this organ.

Leiomyoblastoma in the lower animals is more common than the literature would indicate. In Fox's case the tumor occurred in the uterine cornua and fimbria of an elephant. Malvicini ⁸ reported a fibroleiomyoma in the uterus of a lioness and Frohner ⁹ reported three cases in which the tumors affected the small intestine of a horse. Coca ¹⁰ reported a diffuse leiomyoma of the stomach which resulted in rupture of the organ. My own collection contains leiomyoma from the oviduct of chickens, from the uterus of a cow and several from the wall of the cecum of dogs.

REPORT OF CASE

The animal was an old cow. The previous history was not available. At necropsy a large tumor was found in the spleen and numerous nodules of various sizes were distributed over practically the entire liver. No other demonstrable lesions were found although careful search was made of all internal organs.

Gross description of the spleen: The tumor which was situated near one end of the spleen was firmly embedded in the substance of the splenic tissue. The mass was oval and was raised above the free surface of the spleen. The serosa was not ruptured but invested the growth in a taut glistening covering. In sharp contrast

to the color of the splenic tissue the tumor was yellowish white. It measured 9 cm. by 4 cm. The neoplastic tissue was firm and was separated from the splenic substance by a rather sharp line of demarcation (Fig. 1). A small secondary nodule was present in the splenic pulp just outside the major portion of the growth.

Pathologic histology: The splenic tumor was invested with a flattened capsular structure which consisted of a mixture of fibrous and smooth muscle tissue suggesting that it was a continuation of the splenic capsule. The neoplastic elements were simple, consisting of strands of tissue the various collections of which were laid down at different angles (Fig. 2). It was impossible to discern the cellular outlines although the nuclei were sharply defined. In cross-section the nuclei were circular, while longitudinally they were slender and elongated, the average being about six times longer than their diameter. The nuclei were fairly constant in width throughout their length, with abruptly formed, rounded ends predominating. Many of the nuclei near one extremity revealed what appeared to be a nucleolus. An abundance of finely granular chromatin material was present. Mitosis was common (Fig. 3). The intercellular material was fine, fibrillar and wavy, running parallel to the longitudinal axis of the nuclei. Sections stained by Van Gieson's method showed a few wavy fibers that gave the color reaction of fibrous connective tissue. These ran in the same direction as the other smaller fibrils which constituted most of the intercellular substance. This stain showed that, aside from the capsule, there was little connective tissue present. Many areas showed evidence of considerable necrosis which was mostly of a coagulative variety. The tumor possessed a generous blood supply, the vessels ranging from minute channels to small arteries. The blood supply was irregularly distributed and the structure of the vessels was characterized by simplicity; it consisted of nothing more than a layer of endothelium resting on the surrounding neoplastic elements.

Gross description of the liver: The liver was slightly enlarged and its external appearance was striking. The surface was irregularly roughened by a large number of nodular swellings of variable size measuring from minute foci to masses 7 cm. in diameter. They were whitish yellow and rather firm. Cross-sections through different areas of the organ showed the nodules to be distributed promiscuously throughout (Fig. 7). The hepatic tissue appeared com-

pressed, due to the pressure exerted by the tumor, and most of the larger vessels were compressed from the same cause.

Pathologic histology: Sections of the liver were obtained from several areas, and while there were minor differences the structure for the most part was a duplication of the splenic tumor. The hepatic foci were perhaps more cellular than in the splenic tumor and mitosis was more abundant (Fig. 4). The capsule remained intact and immediately beneath it there were compressed rows of atrophied hepatic cells running parallel to the capsule. Neoplastic tissue was promiscuously infiltrating the hepatic substance in every direction. Many new bile ducts were present in areas quite devoid of other hepatic elements. A few small areas of necrosis were present but in no sense was this change as extensive as in the splenic material. The blood supply was similar in character to that of the splenic growth. By Van Gieson's stain the same type of reaction was observed as in the sections of the spleen. The fibrous connective tissue fibrils were few in number, wavy in character, and larger than the other fibrils which gave the Van Gieson reaction for smooth muscle elements (Fig. 5). In some parts of the liver the tumor tissue showed signs of regression, the cellular constituents being atrophic and densely arranged. Practically no normal hepatic cells were found in any of the many sections examined from this organ; all were atrophic and flattened by the pressure exerted by the neoplastic tissue which pushed into the hepatic substance from many directions (Fig. 6).

DISCUSSION

According to Smith and Rusk, the tumors capable of arising from the tissue elements of the spleen fall into three main groups: (1) fibroma and fibrosarcoma from the capsular and trabecular framework; (2) lymphoma and lymphosarcoma from the lymphoid elements, and (3) those from vascular or sinus endothelium. In view of the histology of this organ the possibility of a smooth muscle tumor should be considered. Smooth muscle cells are present in the vessel walls and in the capsule and septums in association with fibroblasts. My observations agree with those of Jordan,¹¹ who states that the splenic capsule of the ox has an abundance of smooth muscle. The trabeculae also possess a large amount.

The foregoing facts together with the morphologic features of the

tumor reported here would seem sufficient to justify the designation of leiomyosarcoma. The reasons for assuming that the tumor was primary in the spleen with the liver affected metastatically appear convincing. The splenic tumor was limited in its development to one focus while the hepatic tumors were extensively distributed throughout the organ, as might be expected in a case of malignant primary splenic tumor which metastasized by way of the blood stream, the tumor cells reaching the liver by way of the splenic and portal veins. If the tumor cells in the portal circulation were abundant and commonly present they would, as a consequence, find lodgment quite generally throughout this organ.

Anatomically the structure of the liver makes it practically impossible for metastasis to occur from one lobe to another. Consequently, when all lobes of the liver contain numerous foci the point of primary origin of the tumor must be sought elsewhere. In the absence of lesions apart from the spleen in this case, the spleen must be considered responsible for the histogenesis of the tumor.

The scarcity of splenic lymphatics emphasizes the importance of the blood stream as a vehicle for the transportation of malignant cells both from and to the spleen. Primary splenic malignancy would appear metastatically first in the liver, where it would be likely to be confined unless the cells as a consequence of vigorous proliferation broke through the lobular capillaries and were carried by the hepatic veins to the posterior vena cava, in which case the lungs would also receive them.

The scirrhus change described as being present in certain of the sections of the tumor from the liver is mentioned by Mallory as one of the retrogressions likely to overtake a leiomyoblastoma. This is due, in Mallory's opinion, to interference with nutrition, as a consequence of which the more delicate smooth muscle cells perish while the more resistant fibroblasts persist although often atrophic.

SUMMARY

From a review of the literature pertaining to splenic neoplasms in man and the lower animals it is apparent that such tumors are seldom seen. In the primary splenic tumors reviewed, fibroblastoma, lymphoblastoma and endothelioma were the varieties encountered. Although the histologic elements for such a tumor are

present in the spleen no case of primary leiomyoblastoma of this organ has been previously described so far as can be determined from the literature reviewed. A case of primary leiomyosarcoma of the spleen of a cow with extensive multiple metastasis to the liver is reported.

Note: Owing to the tissues being fixed in formalin it was impossible to apply specific stains for the demonstration of myoglia fibrils.

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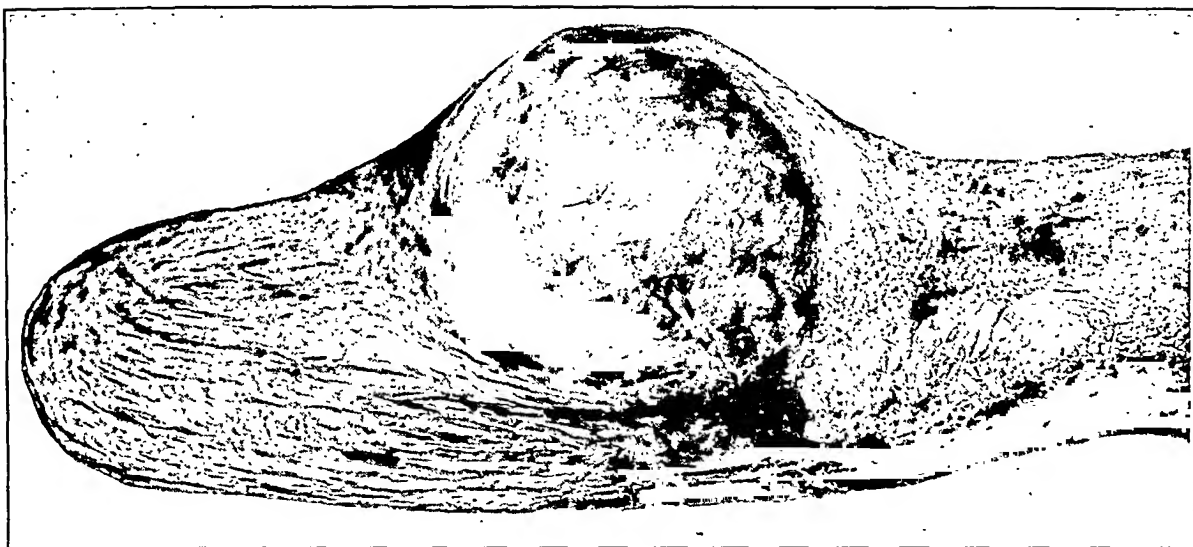
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DESCRIPTION OF PLATES

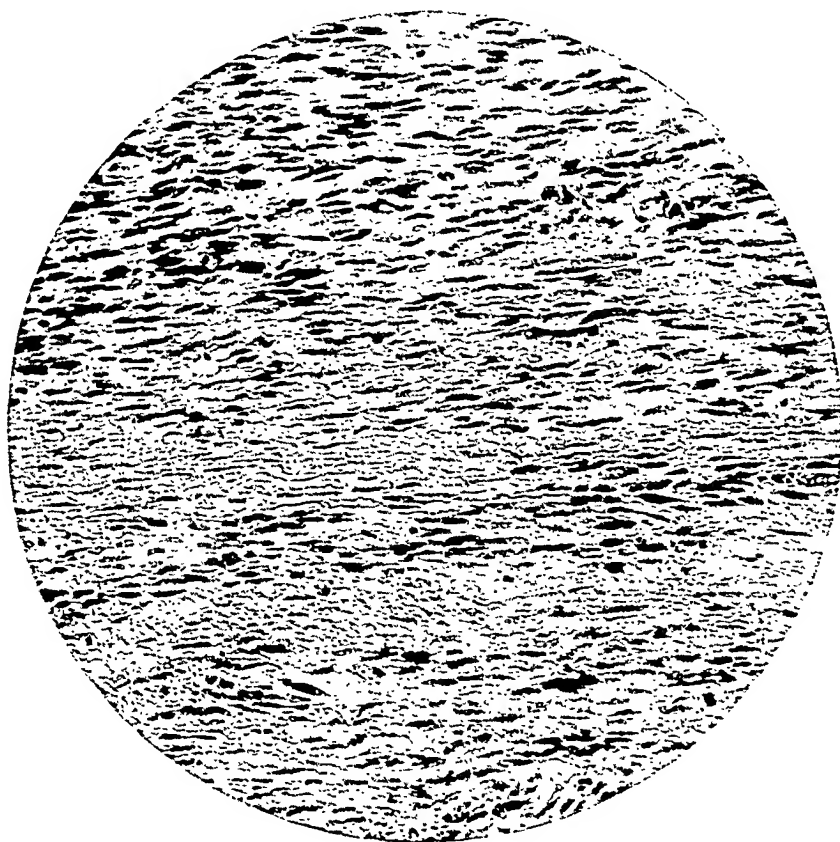
PLATE 33

FIG. 1. Primary leiomyosarcoma of the spleen showing the position of the tumor and its relative size.

FIG. 2. Leiomyosarcoma of the spleen. Simplicity of structure is shown. $\times 150$.



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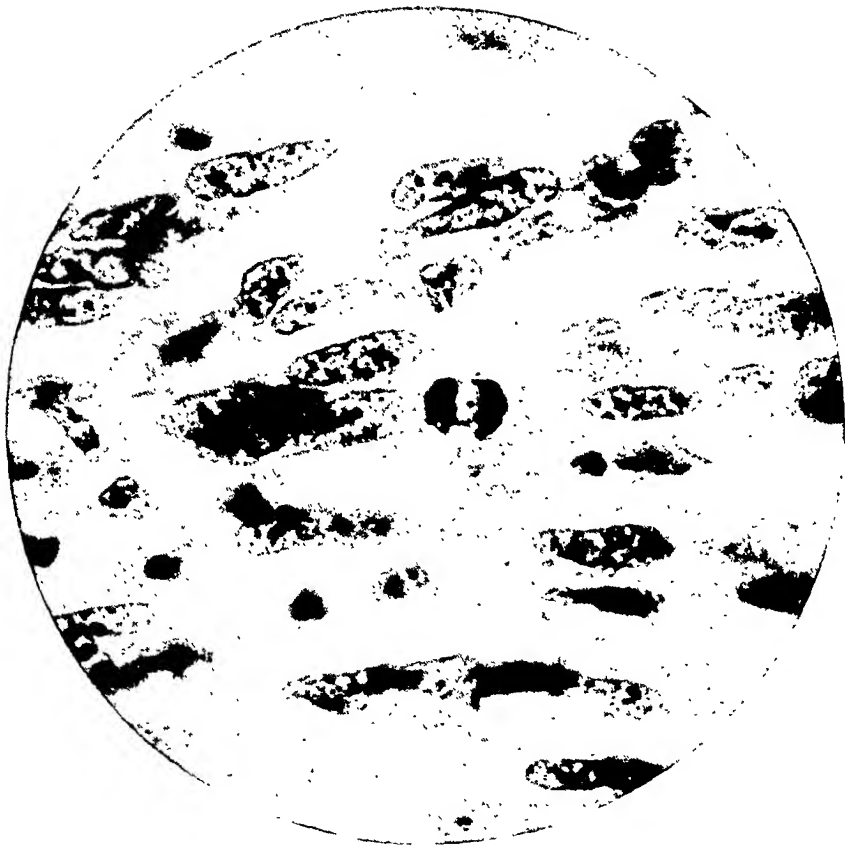


2

PLATE 34

FIG. 3. Leiomyosarcoma of the spleen. One cell in mitosis is shown. $\times 950$.

FIG. 4. Metastatic leiomyosarcoma of the liver. Several cells in mitosis are shown. $\times 770$.



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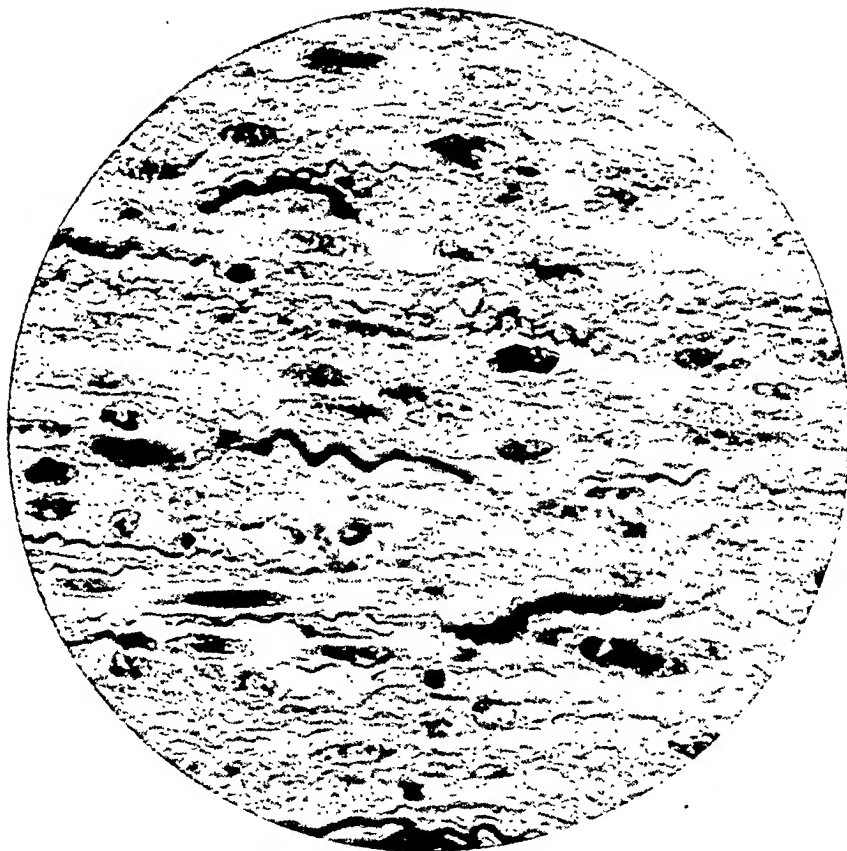


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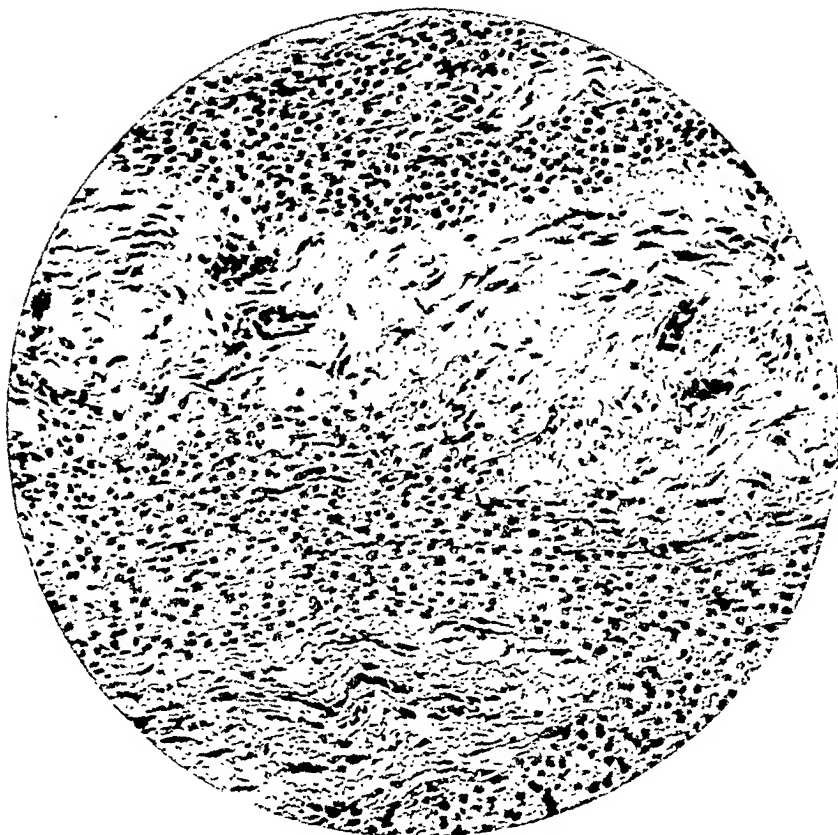
PLATE 35

FIG. 5. Metastatic leiomyosarcoma of the liver. The wavy dark staining connective tissue fibrils are apparent. Van Gieson's stain. $\times 490$.

FIG. 6. Metastatic leiomyosarcoma of the liver. The remaining hepatic cells are flattened and atrophic while the neoplastic elements show evidence of scirrhous change. $\times 175$.



5



6

PLATE 36

FIG. 7. Metastatic leiomyosarcoma of the liver. Cross-section shows extensive involvement of the organ by metastatic nodular masses.



7

Feldman

Leiomyosarcoma of Spleen in a Bovine

OBSERVATIONS ON THE HISTOLOGY OF THE TUMORS OF THE NERVUS ACUSTICUS *

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For many years the proper classification of the circumscribed, encapsulated, slowly growing tumors involving the sheaths of the central and peripheral nervous systems has been a subject for discussion. This group of tumors includes the so-called dural endothelioma or meningioma, the acoustic neuroma, the solitary fibroma of peripheral nerves, and multiple neuromas or Von Recklinghausen's disease. Some authors have felt that each of the foregoing represented a distinct entity. Others, notably Cushing,¹ have advanced the opinion that all should be included under one heading.

Thorough descriptions have been written of all the neoplasms. The solitary tumors of the eighth cerebral nerve, the so-called acoustic neuromas, have received in recent years rather less attention than the other newgrowths of this general class. Pathologists have not agreed as to their origin, and therefore, the type cell and the proper classification have been open to question. For these reasons it was thought worth while to subject a series of acoustic nerve tumors to careful microscopic observation with varied histologic technic to determine if possible in what general class they should be placed.

The tumors of the acoustic nerve have been classified in a variety of ways. They have been called gliomas, neuromas, endotheliomas and fibromas. The authors with conflicting opinions may be separated into two main classes: first, those who consider the growths to be derived from nervous tissue and second, those who hold that they take their origin from connective tissue.

The adherents of the first theory draw their deductions from three lines of reasoning, the first being simply that the microscopic appearance of the tumors is not typical of that seen in ordinary fibromas. They state that their peculiarities of structure, such as whorl formation and palisading of nuclei, are not seen in fibromas.

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From that fact they argue that the tumor is one of a different sort of cell, probably neuroglial in nature.

The French investigators, Lhermitte and Guccione,² represent the second point of view. They stained sections of these tumors with Victoria blue and demonstrated fibrils which colored sharply. As this dye stains neuroglial fibrils well they supposed that this evidence was proof of the origin of the tumors from neuroglial cells. It has been found, however, that Victoria blue stains fibroglia fibrils and fibrin as well as the fibrils of neuroglia. In a publication by Roussy, Lhermitte and Cornil³ the view was also advanced that the tumors are gliomatous in nature.

The third argument is a very important one based on the embryologic work of Harrison,⁴ and Harvey and Burr.⁵ The first showed that when the neural crest is removed from frog embryos of a certain age peripheral nerves develop without a sheath of Schwann. Harvey and Burr demonstrated that the brain of an amblystoma embryo transplanted without the neural crest into a second embryo developed without the formation of a pia-arachnoid. If the neural crest was transplanted with the brain the leptomeninges developed normally. Thus there is strong evidence that the sheath of Schwann and the pia-arachnoid are derived from the neural crest and are ectodermal in origin. Verocay⁶ suggested that the tumors of the acoustic nerve might arise from the sheath of Schwann and applied the term "Schwannoma" to them. If this view is accepted it follows from the work of the embryologists that the tumors should be regarded as of ectodermal origin.

This line of reasoning is open to attack on three grounds. First, Weed⁷ has demonstrated that the pia-arachnoid forms in transplanted embryo brains only when choroid plexus as well as neural crest is included in the transplant. The failure of Harvey and Burr to grow meninges on a brain transplanted without neural crest may have been due as much to the absence of choroid plexus as of neural crest.

The second reason for doubt is the claim made by many authors, notably Mallory⁸ that the tumors arise, not from the Schwann sheath but from the perineurium. This structure is considered fibroblastic in nature by most anatomists. Penfield⁹ speaks of impregnating the fibers of the perineurium by a specific silver carbonate technic for staining collagen.

A third explanation is that the neural crest may contain undifferentiated mesenchymal cells which might give rise to the nerve coverings. There is a group of anatomists who maintain that connective tissue may develop from ectoderm as well as from mesoderm.

The opinion that these tumors are fibroblastic in nature was early voiced by Cushing, who stated that the type cell was probably a transition between neuroglia and connective tissue. Mallory took a very decided stand in favor of the fibroblastic origin of these neoplasms. He demonstrated the presence of fibroglia and collagen fibrils and felt that they should be classed with the tumors of peripheral nerves under the heading of perineural fibroblastoma. His opinion was that both of these growths arose from the fibrous perineurium.

Penfield employs the term perineural fibroblastoma as the result of careful studies using the various specific silver impregnation methods of the Spanish school of neuropathologists as well as routine stains. He states that he has never seen evidence of nervous or neuroglial elements in this type of tumor. This would seem to be a very definite reason for excluding these growths from the class of neuromas or gliomas. He does not state whether he has seen fibroglia or elastic fibrils in these growths although he does mention that he has frequently observed them in the dural endo-theliomas.

In general, five criteria are used to prove that a given tissue is derived from the fibroblast. These are: (1) the morphology of the cell; (2) the presence of collagen; (3) of elastic fibrils; (4) of fibroglia fibrils, and (5) of reticulum. The last may need some explanation. Mallory and Parker¹⁰ have recently shown that the fine fibrils impregnated with reduced silver in the various methods for the staining of reticulum are not fibrils of a specific nature. They are simply very fine strands of collagen which are separated enough by cells or by edema to allow penetration by the silver. Thus the formation of reticulum by a tumor means only that the cells form delicate, discrete fibers of collagen.

A tissue which can be shown to fulfill all of these requirements would be considered by most histopathologists to be fibroblastic in nature. We shall take up these points in order and show that all of the necessary constituents may be found in the tumors in question.

Technic: Fifty-four specimens were examined. All came from

the surgical clinics of Dr. Harvey Cushing. A few were sent to Dr. Mallory years ago from the Johns Hopkins Hospital and the remainder were obtained since then from the Peter Bent Brigham Hospital. Both Zenker's fluid and formalin were used as fixatives. The most important detail in this and other studies is to have absolutely fresh tissue cut into very thin pieces and placed immediately in a large amount of Zenker's fluid. The container should be shaken frequently to insure the contact of fresh fixative with the specimen.

The tissues were embedded in paraffin and cut at six microns. The sections were stained with eosin-methylene blue, phosphotungstic acid hematoxylin, Mallory's aniline blue collagen stain and neutral ethyl violet-orange G. Both the silver carbonate and silver oxide methods of Foot^{11,12} were employed for staining reticulum. Bielschowsky's method for neurofibrils was used. The elastic fibrils were stained by the methods of Verhoeff and Weigert.

Morphology: As described by Cushing the tumors are composed of two types of tissue, one made up of dense interlacing fibrous bands and the other of loose reticular tissue. The first type is composed of a mass of elongated cells with oval nuclei of moderate size containing a rather small amount of evenly distributed chromatin. Palisading and whorl formation of the nuclei are often well marked. Between the cells and parallel with them run innumerable delicate wavy fibrils which take a light brown color with phosphotungstic acid hematoxylin and pale blue with Mallory's aniline blue collagen stain. Their color is pink when stained with eosin-methylene blue. As these fibrils become more closely packed together a darker and stronger brown to red color is developed, exactly like that given by collagen in general when stained by phosphotungstic acid hematoxylin. The reticular tissue is made up of elongated cells of a similar type but more or less separated from each other. The cell processes anastomose, forming a loose network. In the spaces so formed can be demonstrated wandering phagocytic cells, many of which contain fat. The loose tissue is very likely to contain precipitated albumen and fibrin, also at times blood or blood pigment. This tendency to degeneration is very likely to complicate the picture and make the determination of the true structure difficult.

There is a well marked stroma of fairly heavy connective tissue strands carrying with them fair sized vessels. In this stroma isolated fibroblasts are often found which sometimes contain a well

marked nucleolus. It is possible that these have at times been mistaken for nerve cells but we have nearly always been able to demonstrate fibroglia fibrils on them. True nerve cells which impregnate by the Bielschowsky method are extremely rare but are occasionally present. When nervous elements are present they probably have been included in the tumor in its growth since they are entirely different in morphology from the characteristic tumor cells.

Collagen: The background of the tumor is made up of parallel strands of fine wavy fibrils as previously described. When well separated they take a light brown color with phosphotungstic acid hematoxylin but when they are closely packed, the bright red-brown, characteristic color of collagen appears. With the other stains these fibrils take the color of fine strands of collagen.

Elastic fibrils: Only a few of the tumors show elastic fibrils in the tumor itself, although they are common enough in the stroma. When present they are unmistakable. The arrangement is a branching, anastomosing network of fine to thick, irregular strands running at various angles to the predominating direction of the tumor cells. There are a number of fibers of similar shape and arrangement present in these tumors which stain lightly or not at all by the various methods for specifically coloring elastic fibers. They stain sharply by phosphotungstic acid hematoxylin and neutral ethyl violet-orange G. These we take to be poorly developed fibers of the same type.

Fibroglia fibrils: These are the most difficult to find of any of the intercellular products of these tumors. The most exhaustive search was required to find fibers coarse enough to be suitable for photography. Fibrils, staining properly, and of the required morphology and arrangement, were present in abundance in many of the tumors but were too delicate to photograph distinctly.

Reticulum: By proper methods of silver impregnation abundant fibrils may be shown, thickly packed and running parallel to the cell bodies. By careful comparison with preparations stained by other methods they can be shown to correspond exactly to the pale staining wavy fibrils which form the mass of intercellular substance of the tumor and which we have described under the heading of collagen.

DISCUSSION

From the foregoing findings the pathologist would have no hesitation in saying that these tumors are of fibroblastic nature. The embryologic evidence is very hard to explain unless we assume that it does not apply to the development of the perineurium and that the tumors do develop from this structure. Without entering that phase of the subject we can say only that by all the ordinary criteria the tumor is fibroblastic in nature. If the neural crest gives rise to the perineurium as well as the sheath of Schwann it may be possible that undifferentiated mesenchymal cells are present in it and from them the perineurium develops. At all events, whatever the embryology may be, the morphology of the cells and the chemical reactions of the intercellular products are those of connective tissue. In view of these facts we feel that they should be classed with the perineural fibroblastomas and not with the gliomas.

SUMMARY

1. The type cell of the solitary tumor of the acoustic nerve, the so-called acoustic neuroma, forms collagen, elastic and fibrogia fibrils, also reticulum.
2. The histologic characteristics of the tumor are consistent with those of newgrowths derived from connective tissue.
3. The development of the characteristic fibrils is not so well marked as in connective tissue growing in other tumors.

CONCLUSION

The solitary tumor of the eighth cerebral nerve, the so-called acoustic neuroma, should be classed as a perineural fibriblastoma.

We are indebted to Miss Lillian M. Leavitt for histologic aid and to Miss Catherine G. Norton and Dr. F. B. Mallory for the photomicrographs.

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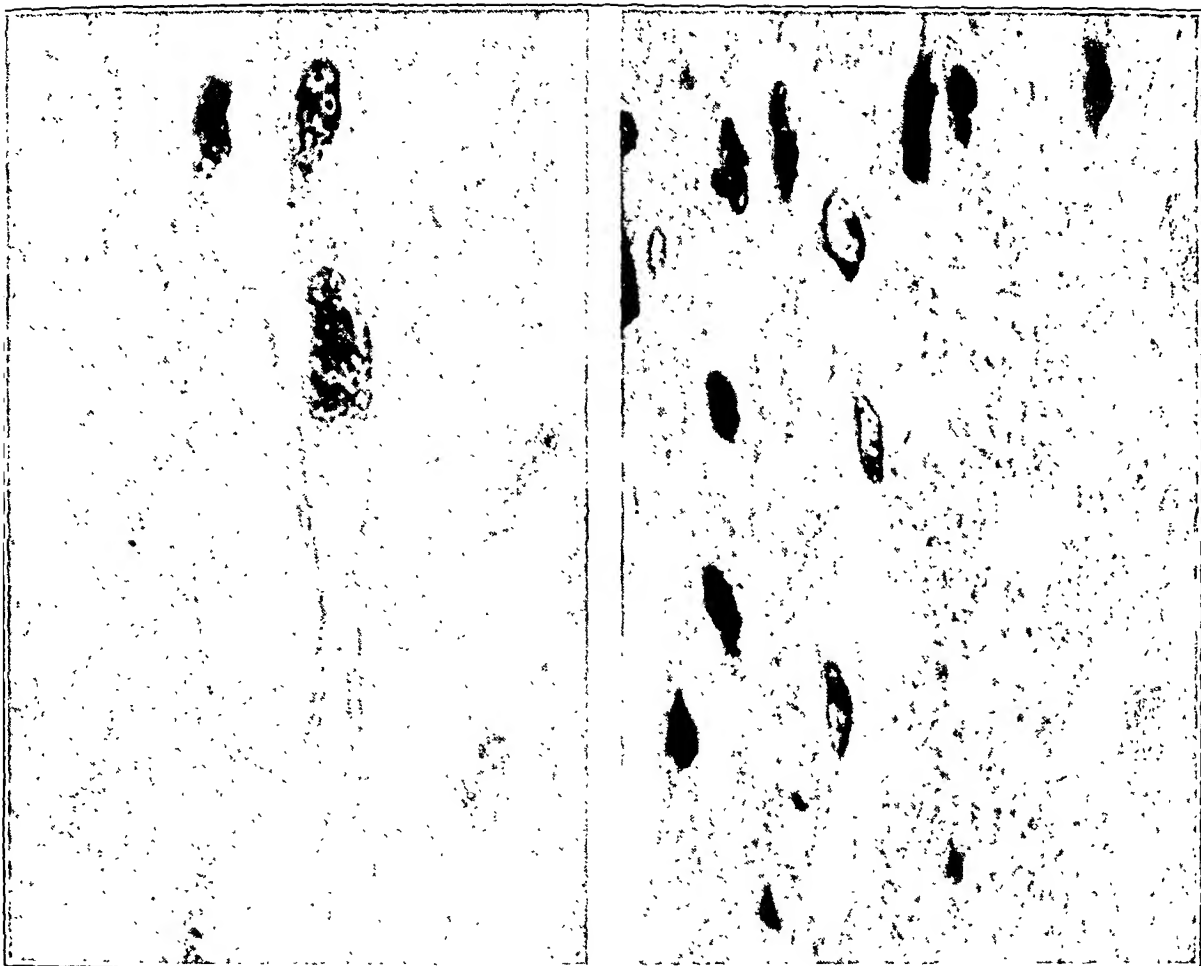
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DESCRIPTION OF PLATES

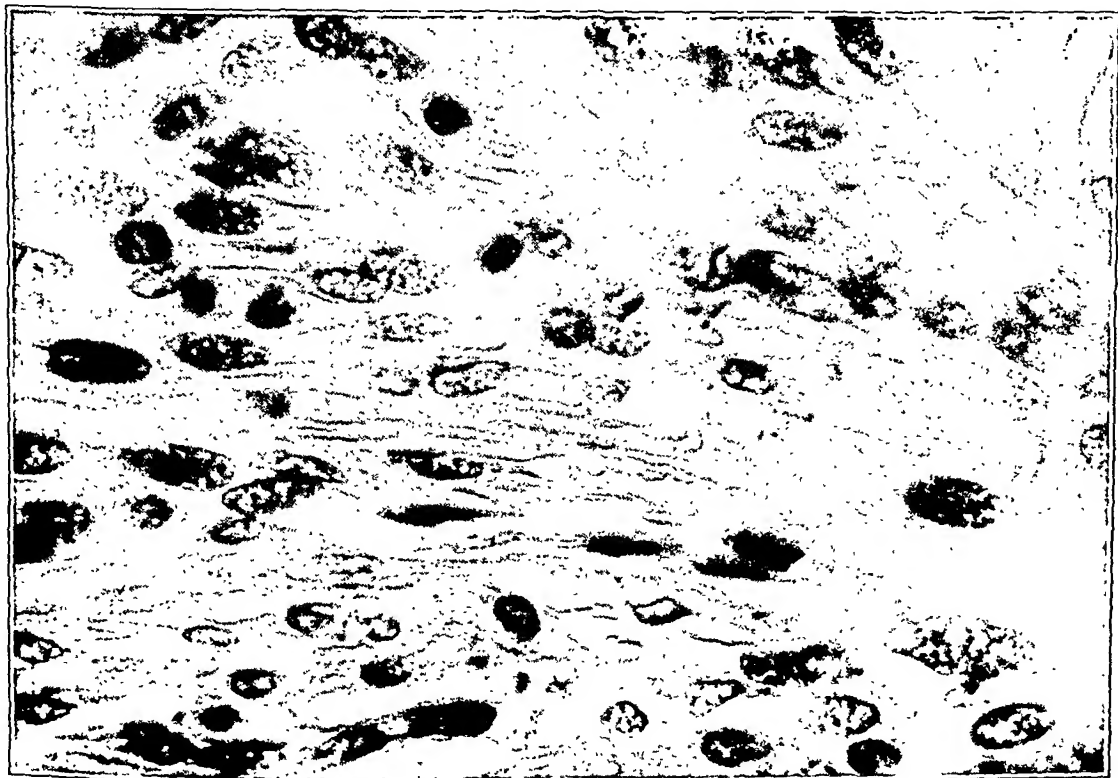
PLATE 37

- FIG. 1. Photomicrograph showing delicate fibroglia fibrils on either side of a characteristic cell of an eighth nerve tumor. $\times 1500$.
- FIG. 2. Section cut across a bundle of tumor cells showing fibroglia fibrils in cross-section. $\times 1500$.
- FIG. 3. Fibroglia fibrils in an arachnoid fibroblastoma for comparison with those in a perineural fibroblastoma. $\times 1000$.



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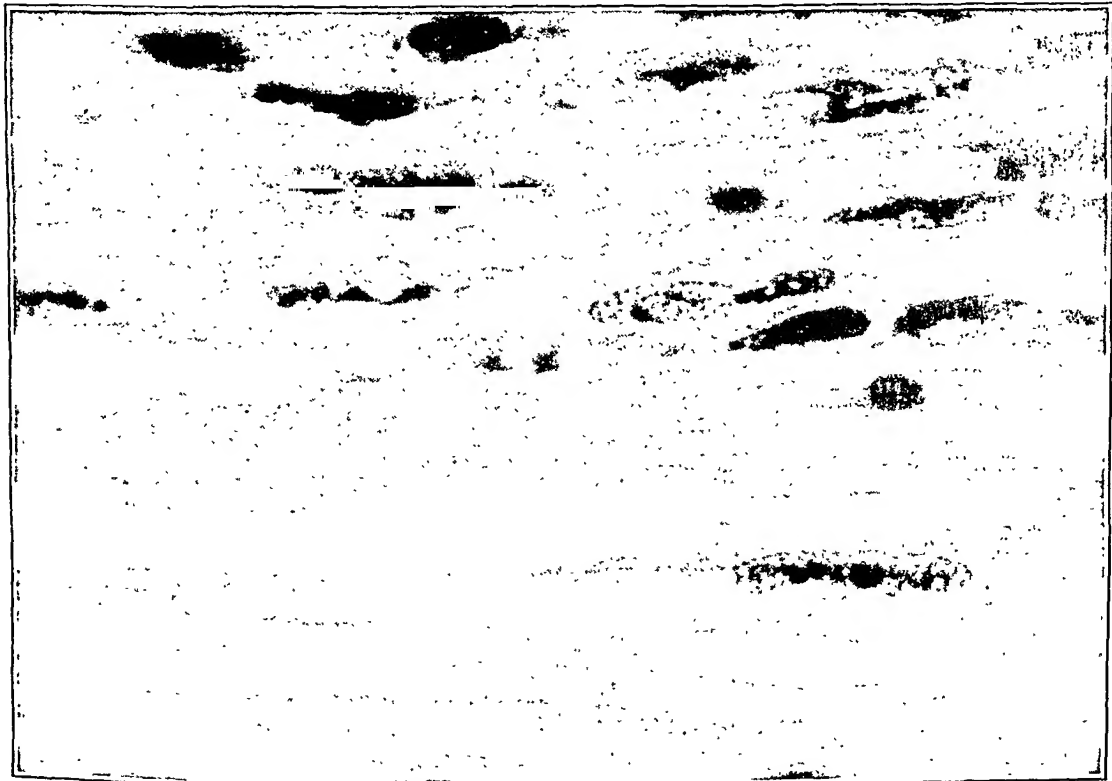
PLATE 38

FIG. 4. Section of a perineural fibroblastoma showing several tumor cells with fibroglia fibrils. $\times 1500$.

FIG. 5. Showing a fibroglia fibril passing over the nucleus of a tumor cell. Other fibrils show less distinctly between the other cells. $\times 1500$.



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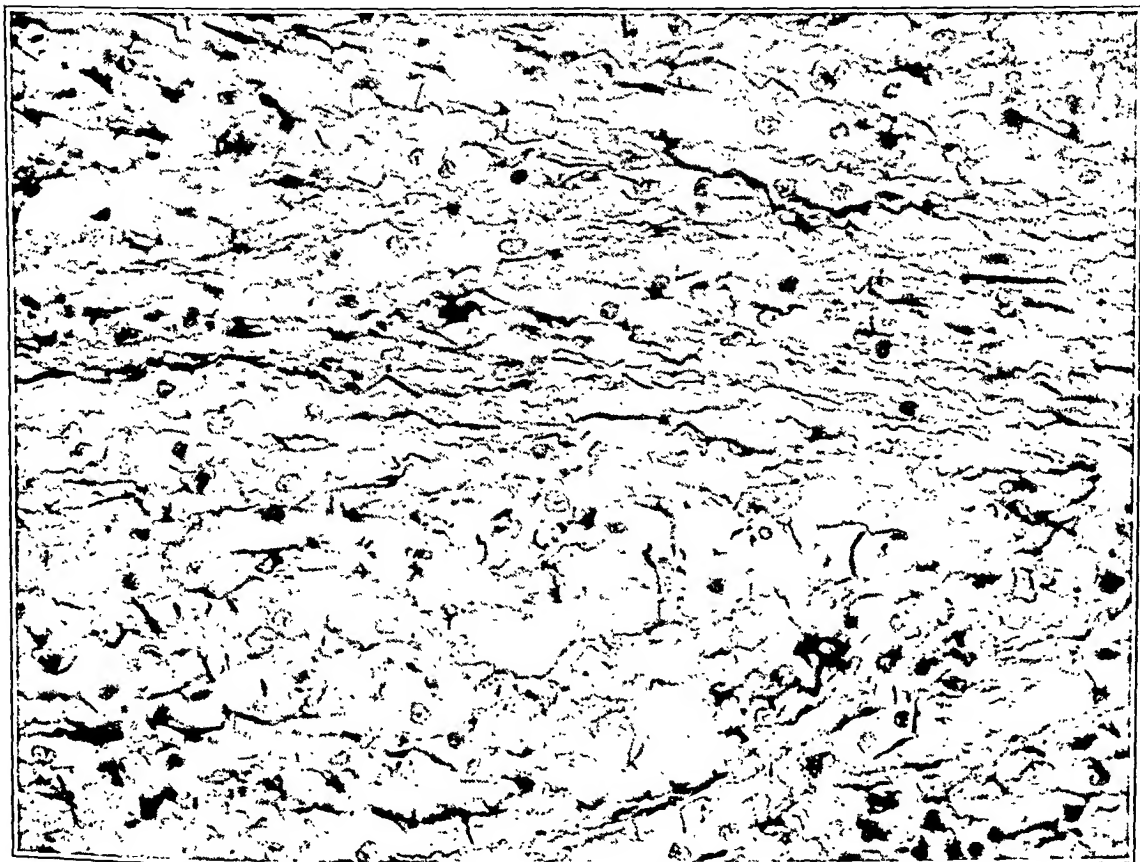
PLATE 39

FIG. 6. Dense network of coarse elastic fibers in a characteristic area of a perineural fibroblastoma. $\times 500$.

FIG. 7. Reticulum in an area of the tumor where the structure is loose and infiltrated with endothelial leucocytes. $\times 500$.



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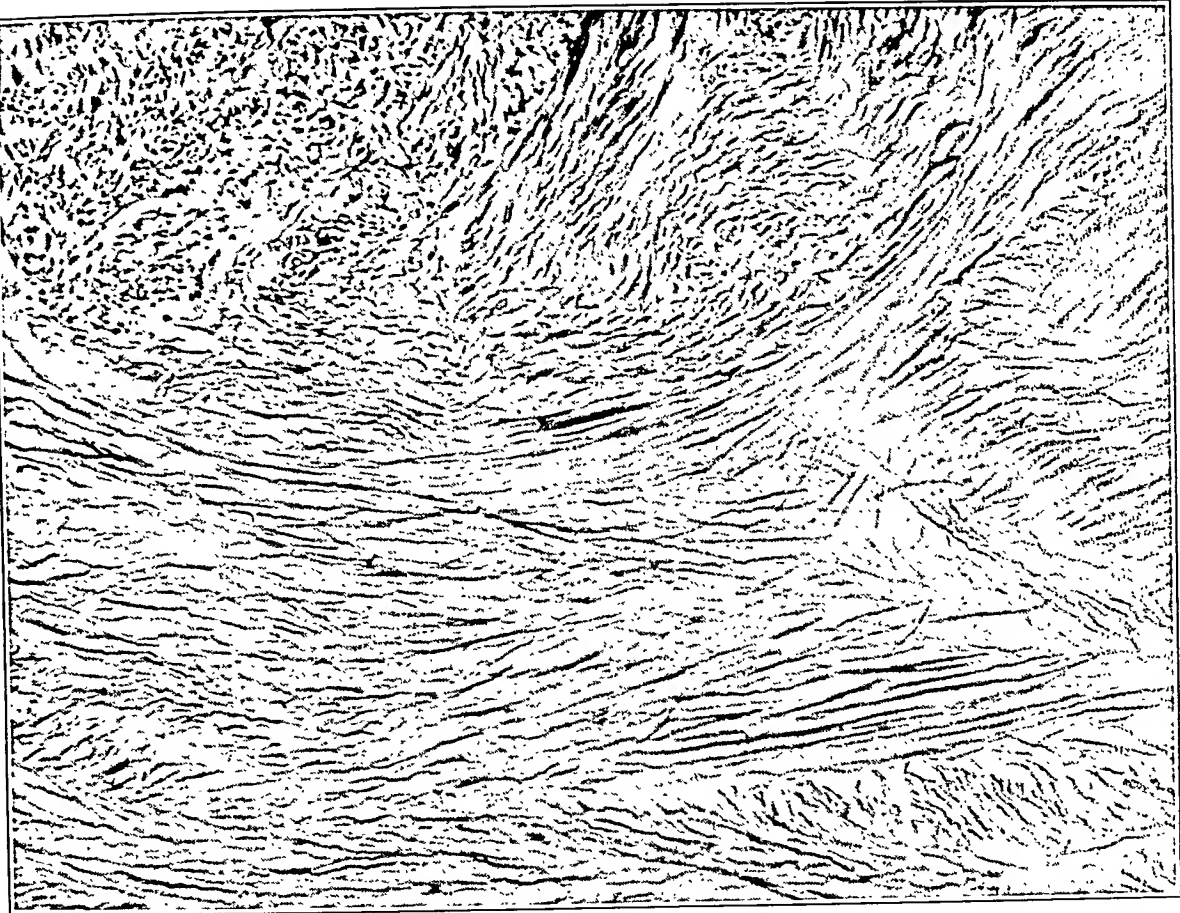


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PLATE 40

FIG. 8. Reticulum in an area of the tumor where the structure is more dense. The fine fibrils which make up the background of the tumor impregnate sharply. $\times 500$.

FIG. 9. Fibroglia fibrils on two fibroblasts in the stroma. The nucleoli are well marked. $\times 1500$.



8



9

A METHOD OF STAINING OLIGODENDROGLIA AND MICROGLIA (COMBINED METHOD) *

WILDER PENFIELD, M.D.

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The smaller cells of the central nervous system were able to hold back the secret of their form for many years in spite of the variety of methods used to stain the other cellular elements. Robertson¹ revealed the cytoplasmic expansions of oligodendroglia with platinum but the method was so unreliable that it never found its way into laboratories outside of Edinburgh.

Most methods stain the nuclei of microglia and oligodendroglia and by Nissl's method the cytoplasmic bodies are often indicated, but not the expansions. Del Rio-Hortega² stained and described microglia completely by silver carbonate and later, by a small modification of the same method, he also revealed the structure of oligodendroglia.³

Silver carbonate may be used to demonstrate microglia (Hortega's cells) even after they have been changed into the rod cell form or transformed still further into compound granular corpuscles.⁴ In this phagocytic state these cells, after being stained with silver, may be readily counterstained by the commoner methods for fat.

When oligodendroglia has undergone acute swelling either during life or as a postmortem change a successful preparation shows the cell body to be swollen with unstained fluid, the expansions to be fragmented and these fragments often similarly distended.⁵ Autopsies must be early to avoid such change in these cells as an autolytic process. But it also occurs during life in various types of intoxication.

The method of Del Rio-Hortega necessitates primary fixation in formalin-ammonium-bromide solution. This is true also of the alcohol modification of this method.³ In a paper before the American Neurological Association in 1925, Globus⁶ provided the key to

* Received for publication January 13, 1928.

the utilization of formalin material for the gold and silver stains which require ammonium bromide as mordant. At that time he proposed a modification of Cajal's gold chloride sublimate method for neuroglia. He removed the excess formalin from the sections with ammonia after the custom of Del Rio-Hortega, but left them in ammonia a longer period and then placed the sections in hydrobromic acid before proceeding with the impregnation. This admirable variation will sometimes yield excellent results even in old material.

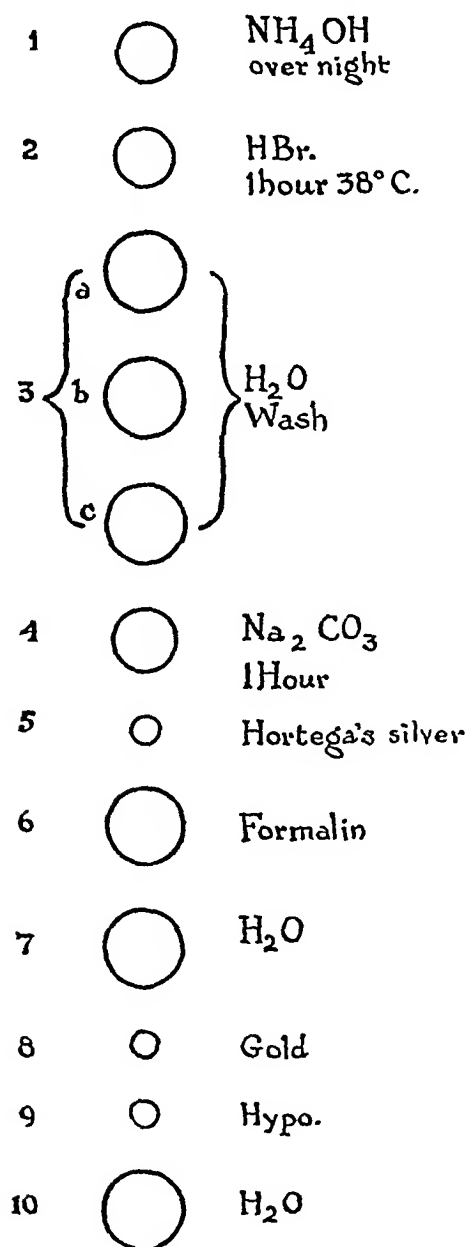
In our laboratory Dr. M. Fulstow began at once to apply this maneuver to Del Rio-Hortega's method of staining microglia and oligodendroglia. She found that successful results could also be obtained for these cells if sodium carbonate be used between Globus' hydrobromic acid and Hortega's silver. In a long series of trial variations during the past year we have standardized the technic as far as we were able.

Consequently, in numerous minor, though important details the method, as it is used at the Presbyterian Hospital, differs from that published by Dr. Globus. A descriptive note, therefore, seems justifiable particularly as the method has proved to be a combined one demonstrating both microglia and oligodendroglia neither of which could be stained in our hands with the procedure outlined by Globus.⁶ Moreover to judge by the number of inquiries which have reached us concerning the technic of demonstrating these cells, there is a rapidly growing interest in them.

The results by this method are not better than those obtained by the original Hortega methods when the latter are successful. But the new procedure is more consistently successful with pathologic material and primary fixation in formalin provides tissue for other stains. Very good results are obtained if blocks be taken from the brain after hardening for five days in 10 per cent formalin. The staining is better and the cytologic preservation improved if the brain is originally injected with formalin. Merck's neutral blue label formalin gives better results than some other formalins in our hands. Doubly distilled water should be used for all solutions and the glassware must be clean.

If successful, both types of cells are well stained throughout but inequality in the perfection of impregnation throughout a single section may be seen. For example, microglia may stain best in

the gray matter and oligodendroglia in the white matter. Because of such variations in a single preparation, sections may be mounted after varying lengths of time in silver. It is of cardinal importance



TEXT-FIGURE 1

that the worker carrying out the technic shall be able to recognize the cells in question on the wet slide before mounting and that he be familiar with the typical pathologic alterations of these cells.

THE METHOD

1. *Harden*: Tissue in 10 per cent formalin (or formalin-ammonium-bromide) for an indefinite period. About a week in formalin gives excellent results.
2. *Section*: Cut sections at 20 microns on the freezing microtome and receive them in 1 per cent formalin or distilled water. Through the succeeding steps the sections should be handled by a glass rod shaped like a hockey stick.
3. *Deformalinize*: Place sections in dish of distilled water to which 10-15 drops of strong ammonia have been added and cover so as to prevent escape of ammonia. Leave in this solution over night to remove formalin (see Text-Fig. 1, No. 1).
4. *Bromurate*: Transfer sections directly to Globus' hydrobromic acid in 5 per cent solution (5 cc. of 40 per cent hydrobromic acid plus 95 cc. distilled water). Place in incubator at 38° C. for one hour.
5. *Wash*: Pass through three changes of water (a, b, c).
6. *Mordant*: Place sections in 5 per cent solution of sodium carbonate for 1 hour. (Sections may remain here 5 to 6 hours without ill effect.)
7. *Impregnate*: Pass sections with or without washing direct to Del Rio-Hortega's silver carbonate, weak solution,* and leave them here 3-5 minutes. Sometimes they may be left till they begin to turn a yellowish gray. Then transfer them to the reducer. Control the duration in silver solution by taking out a section at intervals of 1-2 minutes and examining under the microscope. The sections should turn a smooth gray color in the following reducer.
8. *Reduce*: Plunge into 1 per cent formalin and agitate.
9. *Wash*: Distilled water.
10. *Tone*: Leave in gold chloride † (1-500) at room temperature until all yellow tint disappears and the sections are a smooth bluish gray.
11. *Fix*: Hyposulphite of soda (5 per cent photographic "hypo").
12. *Wash*: Distilled water.
13. *Dehydration* may be done conveniently after Del Rio-Hortega's custom as follows: Float sections on to slide and flatten out with needle. Wash with two to four changes of 95 per cent alcohol from a drop bottle. Follow this with a few drops of carbol-xylol-creosote (proportion of 1-15). When clear, drain slide and blot immediately with two thicknesses of fine filter paper. Mount in Canada Balsam.

* Del Rio-Hortega's ammoniacal silver carbonate (weak solution)

Silver nitrate (Merck), 10 per cent solution	5 cc.
Sodium carbonate (pure), 5 per cent solution	20 cc.
Ammonium hydroxide (sufficient to dissolve the precipitate)	
Distilled water, ad.....	75 cc.

The ammonium hydroxide in strong, fresh solution should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. It is important not to add too much ammonia. A fine black sediment may remain behind which does not resemble the more voluminous precipitate of silver carbonate. This fine sediment should be filtered off. The solution may then be preserved in a dark bottle for long periods.

† The yellow variety of gold chloride is less expensive than brown gold and seems to be preferable for toning.

By this method microglia and oligodendroglia may be stained with a considerable degree of consistency. The morphologic differences of the two types of cells make it quite easy to distinguish them. This differentiation is made even simpler when either type of cell has undergone some pathologic change. For example: a generalized toxic condition, or autolytic and postmortem influence may cause acute swelling of oligodendroglia⁵ while microglia is left unaltered.

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AN INFLAMMATORY BASIS FOR CORONARY THROMBOSIS *

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Occlusion of a coronary artery is almost always due to a thrombus or an embolus. Of the twenty-three cases of occlusion reported by Wolff and White¹ nineteen were due to thrombosis and four to embolism. The usual sources of emboli were either a mural thrombus of the left ventricle or a vegetative endocarditis. Thrombosis never occurs in a healthy vessel, while emboli may occlude normal coronary arteries. In Hamman's² review of the relative frequency of thrombosis and embolism, he reports that Longcope had sixteen cases of thrombosis and one occlusion by embolus; that Faulkner, Marble and White had twenty-five cases of thrombosis showing arteriosclerosis and one vegetative endocarditis with embolism; that LeCount had twenty-nine cases of thrombosis and one of endocarditis with embolism.

Wolff and White state that "the most commonly involved vessel is the anterior descending branch of the left coronary artery, but any of the other branches may be the seat of the occlusion." Of their nineteen cases of thrombosis the left coronary was involved in thirteen, the right in four, both in one case; and the remaining one was an unusual case with the occlusion at the root of the aorta. Wearn³ reports that sixteen out of his nineteen cases showed the occlusion in the anterior descending branch of the left coronary, one in the posterior descending branch, and one in the posterior circumflex branch of the left coronary. In the review by Hamman, referred to above, he reports that of thirty cases the anterior descending branch of the left coronary artery was occluded in twenty-two cases, the orifice in two and the left circumflex in one case, while the right coronary artery was involved in five cases. Of course not all of these cases showed thrombosis. Twenty-five showed arteriosclerosis, four syphilitic aortitis, and one an embolus. We see from these records that occlusion occurs most frequently in

* Received for publication January 13, 1928.

the anterior descending branch of the left coronary artery and that the occlusion is almost always due to thrombosis.

In practically every case of coronary thrombosis described there has been generalized arteriosclérosis or at least sclerosis of the coronary arteries. However, in two of Herrick's ⁴ cases the only significant vascular sclerosis was in the coronary arteries. Riesman ⁵ states that the etiology of coronary thrombosis coincides with that of coronary sclerosis. According to Wearn, in addition to the coronary arteries which were markedly sclerosed in every case, there was sclerosis of the aorta varying from mere fatty plaques to extensive atheromatous ulcerations with calcification. Hamman found that the chief cause of occlusion was arteriosclerosis and the final closure was usually due to thrombosis. He concluded that arteriosclerosis alone would cause such gradual obstruction that a compensating collateral circulation would develop so as to prevent alarming symptoms. But when the vessel has become markedly diseased, even before the inner walls have approximated, thrombosis usually occurs to occlude the vessel abruptly. Gordinier ⁶ also mentions the atheromatous changes in the coronary arteries associated with coronary occlusion. Wolff and White state that "the coronary arteries in all cases of thrombosis are sclerosed, and usually narrowed." They agree with Hamman as regards the arteriosclerosis and narrowing of the lumen, and add that "an exceptional case may show only very slight fibrotic changes, but apparently the injured intima in such instances is sufficient to allow thrombosis to occur." Willis ⁷ says that patients dying of coronary thrombosis always show arteriosclerosis of the coronaries. He adds that the aorta is also the seat of disease, consisting of sclerosis, atheroma and ulceration.

The thrombus usually forms at a point where the sclerotic changes have caused considerable narrowing of the lumen. This point is usually at a variable distance from the orifice but is not far from the bifurcation of the descending branch of the left coronary. In fifteen out of Wearn's sixteen cases the thrombus was situated on the site of a contracture in the lumen due to an atheromatous change in the vessel wall. The thrombus was not found in one of his cases but the lumen of the artery was practically closed at one point by an atheromatous thickening. One of Paullin's ⁸ cases showed thrombosis in the anterior descending branch of the left coronary and

another in the posterior circumflex of the left coronary. The thrombi formed over the calcareous plaques caused infarcts near the tip of the left ventricle and the posterior part of the left ventricle, respectively. The occlusion by a coronary thrombus usually occurs at only one point but more than one vessel may be affected.

It appears that in most cases of coronary thrombosis there is an underlying arteriosclerosis forming the basic lesion upon which the thrombus is formed. Little attention has been paid to the immediate changes which precipitate the deposition of thrombus. It is evident that a coronary artery may remain sclerosed for years without thrombosis and there must be some mechanism which eventually incites the deposit of platelets and fibrin.

This phase of the question has been considered, especially in the two cases to be described, in which the mechanism is clearly in evidence. An arteriosclerotic plaque may lead to sudden thrombosis whether it be situated in the aorta or in a coronary artery by reason of the onset of an acute inflammatory change within the plaque. The cause of the acute inflammation which may occur in atheromata is obscure, but the following cases suggest vascular injury about the atheroma, possibly from circulating toxic material derived from an extensive infection, as one agency in its production.

The outpouring of exudate into an atheromatous plaque distends it with serum, fibrin, red blood cells and white corpuscles. In the two cases studied here such an exudate and hemorrhage were very abundant, and they appear to have been superimposed upon some change, vascular or otherwise, which took place within the atheromatous areas not only in the coronary arteries but also in the aorta. Secondary to the inflammation and the distension of the sclerotic patches the fresh thrombus was deposited, effecting in the case of the coronary vessels immediate and complete occlusion.

CASE 1. The first case is a white housewife, aged 65 years who entered the hospital February 27, 1927 with signs of consolidation and fibrinous pleurisy of the lower right pulmonary lobe. The onset of this condition dated back two weeks before admission when the patient had a cold and cough, followed by shortness of breath, a tight feeling in her chest and some pain.

The course was relatively uneventful until March 3 when she complained of a severe pain in the chest extending across the upper anterior portion and into the right axilla.

Four days after admission the physical signs of a non-productive cough, a prolonged expiration with a grunt in association with rales, dullness in the right chest, suppressed breath sounds and a dry friction rub in the right axilla,

were not definitely changed; but the patient appeared more acutely ill. She vomited once. The leukocyte count rose from 9,000 to 11,000. The temperature which had ranged from 99° F. to 103° F. fell to normal during the evening of March 3; and was 98.2° F. at midnight, where it remained.

The following morning at 8.30 the patient appeared to be in pain but was breathing quietly. While she was under observation, her facial expression showed anxiety and suddenly became fixed, drawn and still. There was no gasping and no struggle. Respiration ceased and her pulse and heart beat were imperceptible.

The anatomic diagnoses are: thrombosis, coronary, left descending; atheromatous degeneration of coronary arteries; acute inflammation, atheromatous areas; necrosis, myocardial, acute; bronchopneumonia, acute, suppurative; hydrothorax, right; acute passive congestion, liver; passive congestion, kidneys, spleen, adrenals and mesentery.

Pathologic Findings: Sections through the descending branch of the left coronary show a large atheromatous plaque which has pushed the thickened intima far out into the lumen of the vessel. Within this atheromatous area there are numerous cholesterol crystal clefts which take various shapes, and some of them are actually pulled apart. There is an abundant content of serum within the plaque which has caused a swelling of the entire area with a pushing out of the intima, increasing the stenosis of the lumen. There are occasional leukocytes scattered through the atheroma and also some about the margin of the plaque. About the intimal and the lateral margins of this atheromatous plaque there are numerous small blood vessels, apparently branches of the vasa vasorum, grown about its margins. Large amounts of fibrin and numerous red blood cells are deposited all about these small vessels. This hemorrhage serves further to elevate the intima and thereby obliterate the lumen still more. The endothelial surface is irregularly roughened from injury, and a fresh thrombus is deposited upon it at the site of greatest obstruction. This completes the obstruction of the already narrowed lumen.

CASE 2. The second case is a white woman, aged 69 years, who entered the hospital with a fairly typical history of gall-bladder disease. She came back later with no improvement of her previous condition. Her blood pressure was systolic 170 and diastolic 72. The leukocyte count was 16,000. She was very jaundiced and this increased along with a rise in temperature. The abdomen became very rigid and about the same time the patient passed a large stone 3 cm. in diameter by rectum. She was operated upon the following morning.

The gall-bladder had perforated into the duodenum. Following the operation for removal of the gall-bladder she apparently reacted well. The temperature rose to 102° F. The wound drained a brownish fluid. At four o'clock in the morning on the ninth day after the operation the patient died. The type of death was not known but it was thought to have been very sudden and peaceful.

The anatomic diagnoses are: thrombosis, coronary, left descending; atheromatous degeneration of coronary arteries and aorta; acute inflammation, atheromatous areas; cholecystoduodenal fistula; peritonitis, acute suppurative, encapsulated; splenitis, toxic; passive congestion, liver, lungs, mesentery and intestines.

Pathologic Findings: The intima of the descending branch of the left coronary artery is irregularly thickened and a large atheromatous plaque pushes the superficial layers of the intima out into the lumen. There are numerous cholesterol crystal clefts within the plaque. There is a large amount of serum present which has caused great swelling and an increase in the size of the plaque with a pushing out of the intima, further obliterating the lumen. Within this edematous plaque about the cholesterol crystals there have accumulated numerous cells, many of which are polymorphonuclear leukocytes. This area is softened due to partial solution of the plaque. About the outer margins of the atheroma there are some small blood vessels with hemorrhage and fibrin deposited all about them. The endothelial surface is irregularly roughened due to some injury and a fresh thrombus is attached to the vessel wall. This thrombus completely obstructs the lumen which has already been narrowed.

Within the intima of the aorta there are large atheromatous plaques which cause the intima to stand out in an irregular manner. There are many cholesterol crystal clefts about which are many polymorphonuclear leukocytes and large mononuclear leukocytes, and a great deal of serum which has caused a swelling of the entire area. This raises the superficial layers of the intima. About the outer margins of the atheromatous plaques there are some small vasa vasorum around which are hemorrhage and fibrin. This hemorrhage and the deposition of fibrin serve to increase the volume of the atheromatous area and to extend the superficial layers of the intima still farther out into the lumen. The endothelial surface is irregularly roughened and a fresh thrombus is attached in places to the vessel wall.

DISCUSSION

The acute changes which have taken place in the above described arteries leading to thrombosis are all superimposed upon a pre-existing arteriosclerosis. They have been found in the aorta and in the coronary vessels. The recent changes have been studied as they have occurred in two cases of coronary thrombosis, in one of which they were present also in the aorta.

The large atheromatous plaques form within the intima producing an irregular thickening which pushes out the superficial layers of the intima into the lumen of the vessel and thereby reduces its size. Superimposed upon these atheromatous areas are acute inflammatory changes. The plaques show the typical clefts that have been left by the cholesterol crystals which have been dissolved. About these irregular clefts serum is present in large quantities and this acute exudation has brought about a marked increase in the volume of the plaques. The increase of the volume in the atheromatous areas serves to extend the superficial layer of the intima still farther out into the lumen. In addition to the serous effusion there has been an exudation of polymorphonuclear leukocytes and large mononuclear leukocytes about the crystal clefts and an acceleration of the process of necrosis and softening. In association with the older sclerotic process numerous small vasa vasorum have grown in about the margins of the atheromatous plaques to supply the damaged wall of the vessel. There are large depositions of fibrin and extensive hemorrhages about these small vessels which indicate some injury to their walls. These areas of hemorrhage and fibrinous exudate begin about the small blood vessels and extend in toward the centers of the plaques and even out under the elevated intima. The volume of the plaques is thereby increased and the intima is raised farther and pushed out into the lumen. The acute inflammation and hemorrhage about the atheromatous areas are probably of very short duration.

The atheromatous plaque with its acute exudate and resulting softening, together with the hemorrhages about the outer margins, causes a great outpushing of the superficial layers of the intima into the lumen. The endothelial surface of the vessel becomes injured due to the various changes going on underneath it or to changes due to the circulating blood passing over the distended and irregular

intimal surface as it stands out in the lumen of the vessel. Upon this injured endothelial surface a fresh thrombus forms, which in the case of the coronary arteries further obliterates the lumen of the vessel or causes complete obstruction.

In both the cases described above there was an acute infection; in the one, an acute bronchopneumonia, in the other a suppurative localized peritonitis. The association of such infections with the acute degenerative and inflammatory process going on in the sclerotic vessels suggests a causal relationship. Infection has long been thought of as a possible etiologic factor in arteriosclerosis for one so frequently finds acutely developed fatty patches in the intima associated with various infectious diseases. That infections of various kinds are operative in acutely advancing and extending arteriosclerotic lesions, already existent but probably quiescent, is equally probable.

These two cases clearly demonstrate the fact, to which we have not found reference in the literature, that an arteriosclerotic plaque may suddenly undergo an acute degeneration associated with inflammation and that thrombosis may be the result. Such an exacerbation of an old arteriosclerosis may be general, that is, it may involve all the arteries affected, and in the case of the coronaries may lead to sudden death from an occluding thrombus.

SUMMARY AND CONCLUSIONS

1. Two cases of thrombosis of the left coronary artery are reported.
2. An acute inflammation of the atheromatous plaques appears to have been the immediate cause of the deposition of thrombus.
3. An extensive suppurative process in a person with arteriosclerosis may cause an acute exacerbation of the vascular lesions wherever they are situated, and in the case of the coronaries may lead to a fatal thrombosis.

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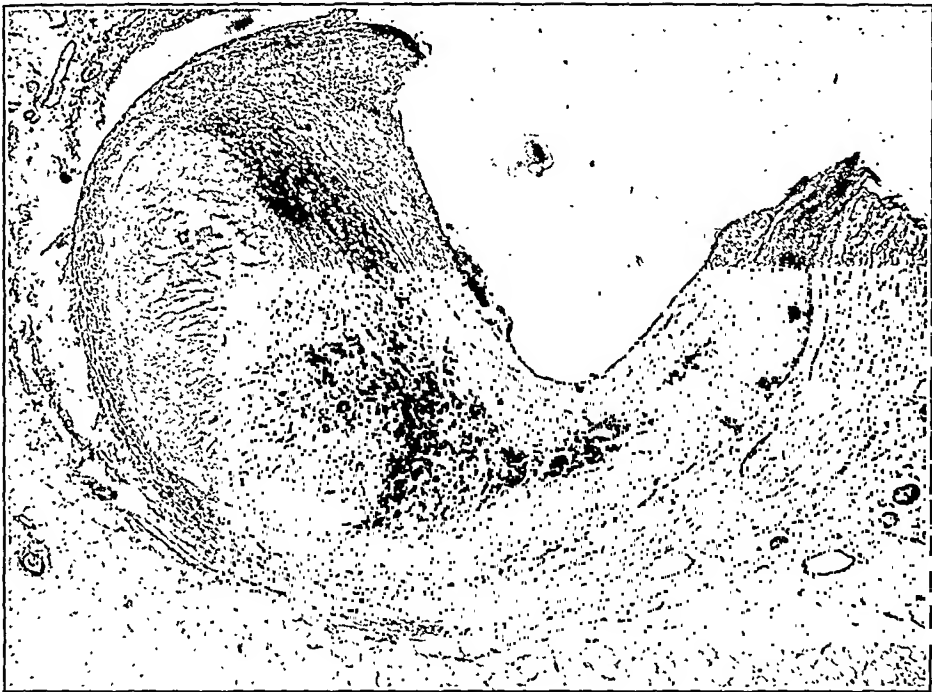
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DESCRIPTION OF PLATES

PLATE 41

FIG. 1. Coronary artery of Case 1. The atheromatous plaque is softened and partially liquefied. The dark areas are composed of fibrin and red blood corpuscles. There is a fresh thrombus attached to the wall of the vessel. $\times 20$.

FIG. 2. Coronary artery of Case 2. The atheromatous plaque is softened and degenerated. The small black dots scattered throughout the plaque are polymorphonuclear leukocytes. The single irregular dark area near the margin is composed of fibrin and red blood cells. There is a fresh thrombus attached to the intimal wall of the vessel. $\times 20$.



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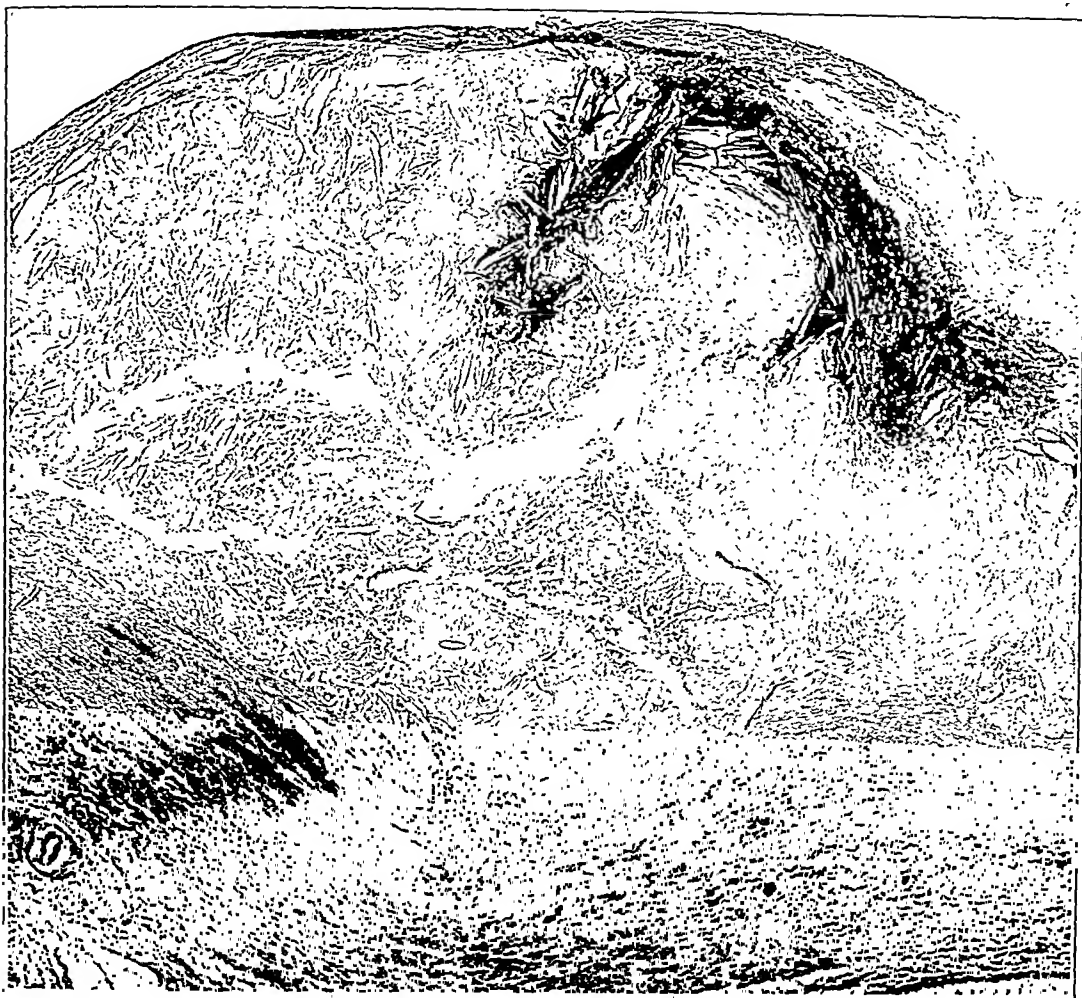


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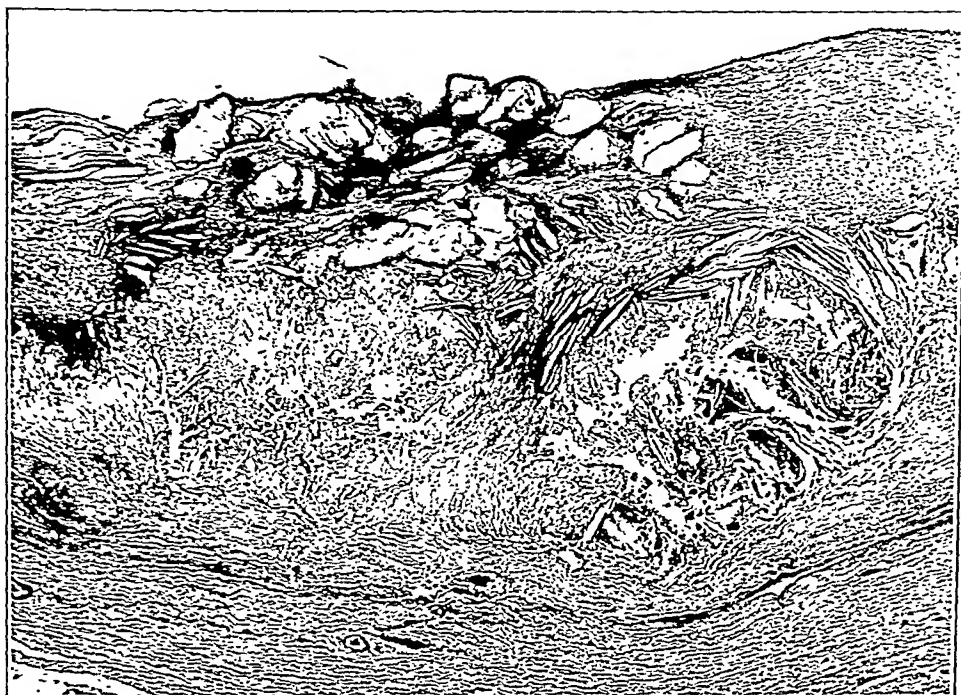
PLATE 42

FIG. 3. Section of aorta of Case 2. The large atheromatous plaque is softened. The dark areas are composed of fibrin, red blood cells and polymorphonuclear leukocytes. $\times 20$.

FIG. 4. Another section of aorta of Case 2. The atheromatous plaque is softened and almost dissolved. The numerous small dots are polymorphonuclear leukocytes scattered throughout. The darker irregular areas are fibrin and red blood cells. $\times 20$.



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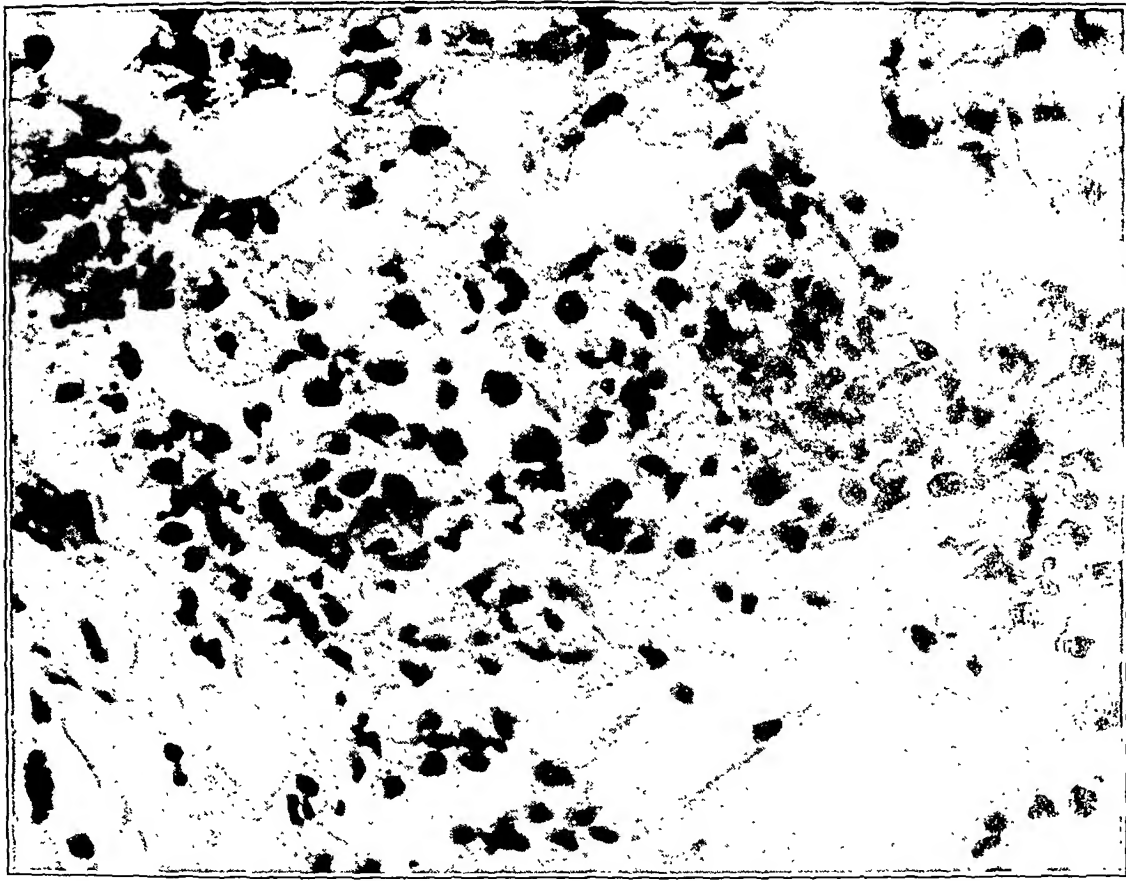


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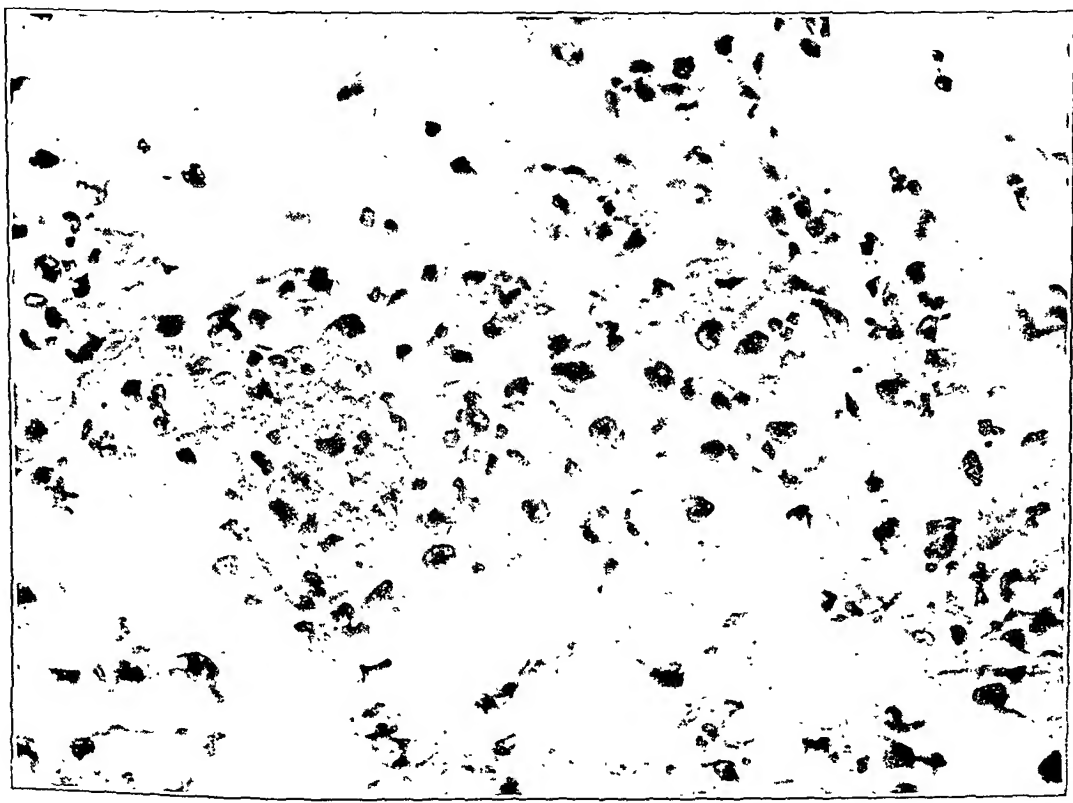
PLATE 43

FIG. 5. A high power view of an atheromatous plaque in a coronary artery of Case 2. The dark cells are all polymorphonuclear leukocytes. $\times 800$.

FIG. 6. A high power view of an atheromatous plaque of the aorta of Case 2. Shows the numerous polymorphonuclear leukocytes scattered throughout in an area of softening. $\times 800$.



5



6

SOME OBSERVATIONS ON INCUBATED LEUKEMIC BLOODS *

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The observations reported in this paper were made during the course of a series of studies of human and animal bloods, exudates and tissues. The purpose of these studies was to gain additional information concerning the origin and physiological activities of the mononuclear phagocyte about which so much has been written in recent times. Leukemic blood was chosen for one set of experiments because of its content of certain types of cells in large numbers. Cultures of normal blood have been reported in detail by M. Lewis,¹ W. Lewis,² Carrel and Ebeling,³ while Awrrow and Timofejewskij⁴ described cultures of blood from cases of myelogenous leukemia. Our results agree very closely with those of the last mentioned authors. Since we were able to employ a more modern and varied technic for the study of the cells we felt that it was worth while to describe our observations. We have not used the word culture in describing our work for we did not have sufficient evidence that the cells under the conditions of our experiments did more than merely survive without undergoing multiplication.

TECHNIC

The blood was obtained in every instance from the median basilic vein. Supravital preparations and fixed smears were generally made before incubation.

The majority of workers in culturing blood have used a solid medium, usually clotted plasma. The drawback to such a medium is that in any one specimen the cells can be observed only once after staining. We felt that if a fluid medium could be employed from which samples could be repeatedly removed the results obtained would be more satisfactory and uniform; the cells would come from the same specimen and would be under the same conditions from day to day. A brief description of the methods employed is given:

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1. *Clotted blood.* One or two c.c. of whole blood was put in test tubes, allowed to clot, then rimmed and incubated. This method was used by M. Lewis at times as a control on her coverslip clot cultures.

2. *Citrated blood.* Blood was diluted with an equal volume of one per cent sodium citrate in Locke's solution. If the blood was incubated in this condition the cells died in a short time (one to two days); it was found that if the citrated blood was centrifuged and one or two drops of the sediment added to one or two c.c. of human or sheep serum the cells survived much longer.

3. *Heparinized blood.* Blood was added to a 1:200 solution of heparin in Locke's solution so that the final concentration of heparin was 1:600 or 1:800. As in the citrated blood the cells died rapidly if the blood was incubated in this state. Therefore, with or without centrifuging, one or two drops of heparinized blood were added to one or two c.c. of heparinized human plasma. The cells under these conditions usually did well; they did even better if several (seven to ten) volumes of Locke's solution were added to a combination consisting of equal volumes of heparinized blood and heparinized plasma.

The test tubes used measured 100×13 mm. The tops of the cotton plugs were cut off and the tubes were sealed with paraffin and incubated at 37.5° C.

Specimens for examination were removed with a platinum loop. These specimens were studied in three ways:—the supravital method as described by Sabin, Doan and Cunningham;⁵ by Wright's blood stain; and by Sato and Sekiya's⁶ peroxidase stain on dried smears.

MATERIAL

Eight cases of myelogenous leukemia and seven cases of lymphatic leukemia were utilized in this study. The total leucocyte counts varied from 6,000 to 226,000 in the myelogenous leukemia cases, and from 2,500 to 400,000 in the lymphatic leukemia cases. The patients were at different stages of the diseases and had had various treatment. At this point, we should like to express our gratitude for material to Dr. George R. Minot and Dr. Henry Jackson, Jr. of the Collis P. Huntington Memorial Hospital, Boston, and to Dr. Ralph C. Larrabee of the Boston City Hospital.

OBSERVATIONS

The results of the different experiments in each group were sufficiently similar to allow a composite description. Each case could be discussed separately but this would entail needless repetition and would be of no advantage. Any significant deviation from the average will be noted. The results of the myelogenous leukemia cases will be taken up first.

MYELOGENOUS LEUKEMIA

The methods of treating the blood have been described above. In each instance, tubes of clotted blood were used and in addition one of the other methods. Of the eight cases, five gave best results in the clotted blood tubes, while the remaining three were most satisfactory in the heparinized blood, heparinized plasma tubes. The periods of survival of the cells were the criteria for judging the results. The character of the cells when in good condition did not differ materially in the various media. The description in each experiment is based on the most satisfactory medium for that particular case.

The word "monocyte" is used in the following descriptions for the mononuclear as described by Sabin, Doan and Cunningham. This cell, as described by these authors, in vital preparations shows the following characteristics:— it is a cell with a single round or indented nucleus; the cytoplasm contains near the nucleus or in its hof a collection of rather fine, salmon-pink granules, often arranged around a clear central area; such an arrangement is known as a rosette; mitochondria occur at the periphery of this rosette and around the nucleus. Phagocytosed material is always placed at the periphery of the cell, never in the hof of the nucleus as in the clasmatocyte. It is a fairly actively motile cell, less so than the polymorphonuclear neutrophile and more so than the lymphocyte. This cell corresponds to the monocyte of Naegeli⁷ — a cell larger than a polymorphonuclear leucocyte with a lobed or indented nucleus and a steel or dusty gray cytoplasm containing numerous granules; the great majority if not all such cells in human blood are oxidase positive.

Polymorphonuclear neutrophiles. While many cells of this type are dead after twenty-four hours of incubation, there are still

many perfectly normal in appearance and motility; some contain a few refractile granules and occasionally a globule staining with neutral red; with the Wright's stain such refractile granules or globules appear as round, clear vacuoles. The dead cells as well as the living give a positive result with the peroxidase reaction. After forty-eight hours, while many still appear normal, an increased number over the previous day show refractile granules and vacuoles. A few contain phagocytized material in addition. At seventy-two hours, still more show the changes described above although there are a considerable number of unchanged cells. From that time on the number of living cells decreases as does the number of those of normal appearance. As a rule, no living cells of this type can be found after two weeks incubation. They always give a positive peroxidase reaction; it is interesting to note that they continue to do so even after they are dead and markedly degenerated.

Polymorphonuclear eosinophiles. This type of cell survives constantly longer than the neutrophile. Living cells are found as late as the twenty-first day. They retain their normal appearance throughout life although occasionally they show a small clear vacuole in fixed smears. They undergo none of the degenerative changes seen in neutrophiles but apparently remain typical until dead. Phagocytosis is never seen. The cell does not increase or diminish in size. The peroxidase reaction is always strongly positive.

Myelocytes. Living cells of this type can be recognized for about as long a period as the polymorphonuclear neutrophiles. Whether they survive longer but in so atypical a form that they cannot be recognized is possible, as will be seen under discussion of the "X" and "Y" cells. While they can be followed, an increasing number show in the vital preparations refractile granules at the periphery of the cell; these granules are usually smaller than those seen in the polymorphonuclear neutrophiles. Stained by Wright's, the cytoplasm contains round, clear vacuoles, and in some is entirely vacuolated. The peroxidase reaction remains positive even after death. In a few instances, typical myelocytes are seen containing phagocytized red blood corpuscles. Whether this type of cell enlarges or changes into an unrecognizable form cannot be stated definitely at the present time. One mitotic figure was seen; differential counts made in several instances gave no evidence of the transformation of myelocytes into polymorphonuclear leucocytes.

Lymphocytes. These cells have a period of survival equal to that of the polymorphonuclear eosinophiles. Like them they preserve their normal character throughout their life with the exception of a rare small vacuole in the cytoplasm. In the vital preparations some show a slight increase in size and number of their normal neutral red granules. No change in size of the cell occurs. Phagocytosis is never seen. The peroxidase reaction is always negative.

Monocytes. The recognition of this cell presents the greatest difficulties after a comparatively short time of incubation. As early as forty-eight hours no typical monocytes can be found by Wright's stain. There are cells present with oval to lobulated nuclei and with cytoplasm so filled with vacuoles that its character cannot be made out. Their size is that of a monocyte. Such cells are either oxidase-negative or positive. In the vital preparations at this time the only type of cell resembling a monocyte is a cell with a round or oval nucleus and a rosette of neutral red granules. The remainder of the cytoplasm is filled with large unstained refractile granules of equal size. Phagocytosis is often present and is always at the periphery. This type of cell we have termed the "Y" cell. It can be followed for a number of days. After about a week larger forms of a similar type begin to appear and increase in number while the smaller type decreases. Whether the larger type of cell is derived from the cell supposed to be the monocyte cannot be definitely stated. A further discussion of this large type of cell is taken up below.

"X" Cells. At about the third or fourth day of incubation a type of cell appears which we have termed the "X" cell as its origin is obscure. This cell at first is about the size of a polymorphonuclear leucocyte or slightly larger. In the vital preparations it appears as a cell with one to two (sometimes four) round or oval nuclei. The cytoplasm by the neutral red method appears filled with numerous fine granules varying in color from yellow-red to brown-red; a rosette is often present, arranged around a clear centrosphere. With the Wright stain the cytoplasm varies from a gray-blue to a robin's egg blue and sometimes contains fine pinkish to purplish granules, especially in the vicinity of the nucleus. The nucleus, usually eccentric, is round with a reticulated chromatin and sometimes contains a nucleolus. Occasionally, where two nuclei are present they are connected by a delicate thread of chromatin. In both fixed and vital preparations the shape of the cell varies greatly. It may be

round, spindle-shaped, stellate or very irregular due to numerous pseudopods. Cells of this type are often phagocytic, especially the medium sized and larger forms. The phagocytized material is always situated at the periphery. By the peroxidase reaction this type of cell is strongly positive, weakly positive, or negative. Such positive granules are distributed evenly in the cell and cannot be due to phagocytized material. As the time of incubation increases the number of cells of this type becomes greater. At the same time larger forms identical in character appear. These are highly phagocytic, containing red blood corpuscles, fragments of hemoglobin and occasionally dead leucocytes. Phagocytized material, as in the smaller forms, is never placed near the nucleus but always at the periphery of the cell. This type of cell constantly lives longer than any of the other leucocytes; living cells have been found in one instance after seven weeks. Three weeks to a month is their usual time of survival. The peroxidase reaction of the larger forms is similar to that of the smaller forms — it varies from strongly positive to negative. The size of the largest cells of this type is about seven or eight times the diameter of a red blood corpuscle. The possible origin for this type of cell will be discussed below.

Large "Y" Cells. As stated under the description of the monocytes, about the seventh or eighth day there appears a large cell whose size is similar to a medium or large "X" cell. As time goes on the number and dimensions of these large cells increases, their greatest size being slightly larger than that of the largest "X" type. These cells have one to two round nuclei. In vital preparations they show a rosette of light red granules; the remainder of the cytoplasm is filled with large unstained refractile granules all of the same size. With the Wright stain the cytoplasm is filled with vacuoles. They are often highly phagocytic and, like the "X" cell, the phagocytized material is always placed at the periphery. These cells generally give a positive oxidase reaction but are occasionally negative. Their duration of life is similar to that of the "X" type and they preserve the characteristics given above throughout their survival.

DISCUSSION

The polymorphonuclear neutrophiles and the myelocytes, as far as can be made out, had about the same period of survival and showed similar degenerative changes. That the myelocytes may have been transformed into one of the long-lived "X" or large "Y" types is possible and this would account for their apparent disappearance. The polymorphonuclear eosinophiles and the lymphocytes survived longer than the above two and neither showed any change in size or staining, except that the lymphocyte occasionally showed neutral red granules larger than normal. Neither cell was even seen to phagocyte. The two types of polymorphonuclear leucocytes and myelocytes gave a positive peroxidase reaction even when dead. The monocytes presented a difficult problem as they lost their normal characteristics so quickly. Even after forty-eight hours no typical monocytes could be found with fixed or vital preparations. In one culture in which the number of monocytes exceeded that of the myelocytes, the cells could be followed better than in the others. Here again even at forty-eight hours the only cells recognizable as monocytes were extremely vacuolated and filled with large refractile granules in the vital preparations. Such cells were generally oxidase positive. Whether this type of cell goes on to form the so-called large "Y" cell it is not possible to affirm or deny at the present time.

The "X" type of cell likewise presents a problem insoluble as yet. These cells were identical in every way with those found by Awrorow and Timofejewskij in their cultures. These authors termed them "*Ausläuferzellen*," "*Hypertrophierte Zellen*" and "*Klasmatozyten*" according to their shape and physiological activities. They believed they were all variations of the same type. They felt that the source of this type was the myeloblast of Naegeli or the lymphocyte. The "X" cell resembles no cell seen in the blood under ordinary conditions. The fact that this type appeared about the fourth day and from then on increased in number and size suggests that multiplication or transformation of other cells must have taken place. The possible origins for this cell are the myelocyte, the polymorphonuclear neutrophile or the monocyte. It cannot be an eosinophile as it is phagocytic, often peroxidase negative and never shows eosinophilic granules. Against its lymphocytic origin are its

phagocytic properties and frequently positive peroxidase reaction. In favor of the myelocyte as its progenitor are the character and arrangement of the neutral red granules in vital preparations and its occurrence in large numbers in blood rich in myelocytes. Against the myelocyte is the fact that the "X" cell is often peroxidase negative and is highly phagocytic; reversion of the myelocyte to a primitive type is a possibility. If it is derived from a polymorphonuclear neutrophile this cell must have undergone a complete transformation as regards its nucleus and cytoplasm. As many typical neutrophiles survive for a long time this explanation seems improbable. The varying peroxidase reaction, the property and method of phagocytosis and the neutral red rosette could well be accounted for by a monocytic origin. Against this, however, is the fact that monocytes even after two days incubation are filled with vacuoles. It seems hard to believe that such a cell should twenty-four to forty-eight hours later give rise to a healthy cell with a cytoplasm free from vacuoles. It is possible, of course, that a certain number of monocytes are more hardy than the others and do not undergo such vacuolization but instead differentiate into "X" cells. However, such monocytes must be extremely rare as no definite, non-vacuolated monocytes have been seen after forty-eight hours incubation. As suggested by Awrrow and Timofejewskij the myeloblast may be the parent cell, but against this is the fact that "X" cells occur in incubated normal bloods and tissues (to be reported later). A further possible source is some primitive type of cell that has the power to differentiate along different lines. Such a cell would correspond to Sabin, Doan and Cunningham's reticular cell or to Ferrata's hemohistioblast. The varying oxidase reaction, content of granules and rather primitive type of cytoplasm could be accounted for by assuming its origin from the hemohistioblast. However, hematologists are divided as to whether such cells do exist even in pathologic bloods. The reticular cell of Sabin *et al.* has not been described as occurring in the blood. Some hematologists believe that a certain percentage of the large mononuclears of the blood are endothelial in origin; such cells might be the progenitors of the "X" cells. In favor of such a view are the phagocytic powers, morphological characteristics and negative peroxidase reaction of some of the "X" cells. Opposed to such a theory is the arrangement of phagocytized material. In an "X" cell, the pha-

gocytod material is always placed at the periphery, never next the nucleus, as described for the endothelial leucocytes (clasmatoocytes) by Sabin *et al.* The positive peroxidase reaction of some of the "X" cells is also against an endothelial origin as endothelial leucocytes and the clasmatoocytes of the tissues are peroxidase negative. We hope that studies now in progress will permit a more definite decision as to the source of the "X" cell.

The large "Y" cell with the neutral red rosette and numerous refractile granules may be merely a form of the "X" cell. The "Y" cell's rosette, peripheral phagocytosis, varying peroxidase reaction, size and duration of life would favor this explanation. Against the identity of the two cells is the character of the cytoplasm, the occurrence of one type alone and the time of appearance. The cytoplasm of the "Y" cell is always filled with large refractile granules. The "X" cell usually shows no such granules; if present they are smaller in size and fewer in number. In the "Y" cell the neutral red granules are light red in color, regular in size and are confined rather sharply to the rosette area. In the "X" cell the neutral red granules vary from yellow-red to brown-red in color and in size from very fine to coarse. They tend to occur throughout the cytoplasm. The finest granules are at the center of the cell and the largest at the periphery. As will be seen in the description of the lymphatic leukemia bloods either type may be present in the absence of the other. The "X" cells appear as early as the third day while the large "Y" cells are not usually seen before the seventh or eighth day. In favor of the development of the large "Y" cell from the monocyte are the similarity as regards the disposition of the neutral red, the large refractile granules in the cytoplasm, the varying oxidase reaction and the peripheral phagocytosis. The only objections to such an origin for this cell are the rapid degenerative changes and decrease in numbers of the monocyte during the early periods of incubation, while the large "Y" cell appears in increasing numbers and size from the seventh day on. The sometimes negative peroxidase reaction, the well developed phagocytic powers and the character of the cytoplasm of the large "Y" cells all argue against the myelocyte as its progenitor. The points in favor and against the derivation of the "X" cell from an endothelial cell apply also to the "Y" cell. As with the "X" cells we hope to be able to report more definite data soon.

It is impossible to say why the cells of some of the incubated bloods survived for long periods of time in the clotted blood tubes, while in others the great majority died in a comparatively short time. The number of cells alone cannot be the reason as the cells of one blood with a total leucocyte count of 17,000 lived but a short time while those of the blood with a total leucocyte count of 176,000 lived a long time. Some other factor or factors must play a part. In one experiment where the heparinized plasma was diluted several times with Locke's solution the cells survived much longer than in the undiluted plasma, suggesting that the serum in certain cases may exert an injurious influence.

It is interesting to note that sheep serum although hemolytic and agglutinative for human red blood corpuscles, apparently has very little effect on the leucocytes, since leucocytes in several instances survived for a long time in such serum.

LYMPHATIC LEUKEMIA

The bloods were treated in the same way as those from the myelogenous leukemia cases.

Polymorphonuclear neutrophiles. These cells as a rule lived on an average but five days in the clotted blood tubes. In some instances all died within forty-eight hours. In the tubes where the blood was diluted the average period of survival was ten days; in one instance reaching fifteen days. The various degenerative changes observed in the myelogenous leukemic series held true here also.

Lymphocytes. In the clotted blood tubes the lymphocytes died out as rapidly and in two instances more rapidly than the polymorphonuclear neutrophiles. In the dilute tubes, however, the average time of survival was thirteen days. During this period of incubation the lymphocytes always preserved their essential characteristics, both in the fixed smears and in vital preparations. They often showed some increase in the number and size of their neutral red granules, which in arrangement frequently suggested a rosette. Such a rosette, however, could not be confused in any way with that of a monocyte owing to the shape and color of the granules. Occasionally the Wright stain showed one or two small clear vacuoles in the cytoplasm; these vacuoles were smaller and fewer in number

than those in a polymorphonuclear leucocyte or myelocyte. Some of the cells increased somewhat in size, rarely being about four times the size of a red blood corpuscle. No mitoses were seen nor were lymphocytes with more than one nucleus noted. In following the cells from day to day it was found that the percentage of small dead lymphocytes as compared to the living was always greater than that of the larger forms. The larger cells always showed a proportionately greater number of living forms, but cells of both types persisted during the period of survival.

Monocytes. In one case a few monocytes were noted in the original blood. In another a few cells, possibly monocytes, were found. After incubation, definite monocytes were present. The remainder of the cases showed no monocytes either in the original smears or after incubation. The changes shown by the monocytes during the time they could be followed were the same as in the myelogenous leukemias.

Myelocytes. In one case the original blood contained two per cent myelocytes. The myelocytes could be followed up to nine days. During this time an increasing number showed vacuolization. Their final fate is in doubt as discussed under myelogenous leukemia.

"X" Cells and Large "Y" Cells. In two cases "X" cells were found appearing in one case at the sixth day and in the other at the eighth. They were similar in appearance to those seen in the myelogenous leukemia bloods but differed in that they were apparently all oxidase-negative. In one case several of the cells showed foci of peroxidase positive granules but from their distribution and arrangement it was obvious that these represented phagocytosed polymorphonuclear leucocytes. Large "Y" cells were found also in two cases, one of these being that in which the "X" cells were found. Their characteristics given above under myelogenous leukemia apply here also. Their peroxidase reaction varied from positive to negative. Their periods of survival were seventeen days to thirty-four days. In no case did the number of the "X" cells or the large "Y" cells approach that found in the myelogenous leukemia bloods.

DISCUSSION

The most striking fact in regard to these bloods was the failure of the cells to survive in the clotted blood tubes. All types of cells apparently died in a comparatively short period of time. The average period of survival was five days although it was at times as short as forty-eight hours. The contrast of the period of survival of the lymphocytes in the myelogenous series to that in this series is striking, the average period in the former being twenty days or four times as long. The polymorphonuclear neutrophils in the myelogenous bloods survived in clotted blood usually about two weeks or three times as long as in this series. In the diluted bloods the lymphocytes survived on an average nineteen days and the polymorphonuclear neutrophils eleven days, a marked contrast to the results in the clotted blood. At first it was thought that the number of cells in the clotted bloods was the reason but apparently this could not be the sole explanation as in one case the total count was 2,500 and the cells died in forty-eight hours. Also, in a myelogenous leukemia case where the total count was 196,000 the lymphocytes survived over two weeks in clotted blood. Some other factor must be involved.

It is interesting to note that whereas the "X" cells in the myelogenous series varied from strongly peroxidase-positive to negative, the "X" cells seen in this series were oxidase-negative. The cells from both series were morphologically identical. It seems improbable that the lymphocyte is the parent cell of the "X" cell in these bloods for throughout the period of incubation the lymphocytes always preserved their normal appearance and within limits their size; they showed no transitions to the other type. The large "Y" cells appeared in the two cases in which monocytes were present; this occurrence may be of some significance. In one of these cases myelocytes were present also. The period of survival of both the "X" cells and large "Y" cells was about the same as in the myelogenous series.

SUMMARY AND CONCLUSIONS

Blood from eight cases of myelogenous leukemia and seven cases of lymphatic leukemia was incubated at 37.5° C. Such bloods were incubated as clotted blood and in addition were diluted in different ways. Specimens were removed at varying periods and were studied

supravitality and in fixed smears. In five of the cases of myelogenous leukemia the cells in the clotted blood tubes survived longer than in the diluted blood tubes but in the lymphatic leukemia cases in no instance did the cells survive as long in the clotted blood as in the dilute tubes.

In the myelogenous series the myelocytes and polymorphonuclear leucocytes lived, on an average, two weeks. The eosinophiles and lymphocytes survived about three weeks. The monocytes showed early vacuolization and were followed with difficulty after the fourth day. They may have become the large "Y" cells.

In the lymphatic leukemia series the lymphocytes and polymorphonuclear leucocytes lived, on an average, five days in clotted blood. In diluted blood the lymphocytes survived nineteen days and the neutrophils eleven days. Monocytes were found in but two cases and showed the same degenerative changes as in the myelogenous series. In one case myelocytes were found and could be followed for nine days.

In all the myelogenous leukemia cases and in two of the lymphatic series two types of cells appeared that are not seen in blood under ordinary conditions. The first, called by us the "X" cell, appeared usually about the fourth day and increased in size and number for several days. The longest time of survival was seven weeks. These cells have round nuclei and basophilic cytoplasm, often with pink or purplish granules; their shape is irregular; they vary from strongly oxidase-positive to negative and they are phagocytic. In the supravital preparation they show numerous fine neutral red granules throughout the cytoplasm but also often in addition in a rosette form, frequently with a clear centrosphere; phagocytosis is always at the periphery of the cell.

The other type of cell, the large "Y" cell, has one to two round to oval nuclei and bluish cytoplasm with numerous vacuoles; is usually oxidase-positive, sometimes negative; in the vital preparations it has a large rosette of neutral red and the surrounding cytoplasm is filled with large refractile granules; phagocytosis is always at the periphery; the shape of this cell is usually round. The origin of both these types of cells is at present undetermined.

The methods employed would seem to be suitable for the study of the effect of different substances or conditions on the normal and tumor cells of the blood.

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CARCINOIDS (ARGENTAFFIN-CELL TUMORS) AND NERVE HYPERPLASIA OF THE APPENDICULAR MUCOSA*

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In 1924, under title of "Neurogenic Appendicitis and Carcinoids," I reported the result of studies commenced in 1913 of more than 400 appendices, some healthy, others with pathological history. I showed that the latter contain neuromata, which perhaps explain the pain of chronic appendicitis. These neuromata arise from the nerves of the periglandular plexus; they form after the migration, into the nerves, of cells containing chromaffin and argentaffin granules which are similar to the granules of the chromo-argentaffin cells of the normal epithelium. I showed further that carcinoids arise from certain intranervous argentaffin cells.

Various considerations lead me to believe that the periglandular plexus from which these neuromata arise is not of sympathetic origin but that it depends genetically on certain entodermic cells and that it represents a *placode*, a *neurentoderm*. This opinion has been rejected as heretical, although the observations on which it is based have been confirmed by many investigators; but no one has been willing to review the question as a whole and with the methods which I recommended.

I return to the subject now because I have controlled my former conclusions by the study of 800 more appendices and because, far from contradicting these conclusions, this study has confirmed them and has enabled me to reply to various objections. I shall report the researches in chronological order; in this way the reader will best understand how and why they led to conclusions which at

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first sight seem revolutionary. My warmest thanks are due to my friend, Dr. George F. Laidlaw, who assumed the task of translating this paper for the Journal.

I. CARCINOIDS

I shall not pause here to review the literature that has been inspired by these curious tumors.* Often multiple, they have been found along the entire length of the alimentary canal from the stomach to the rectum; but their favorite situation seems to be the appendix. Although not strictly identical in every detail, nevertheless, their structure has so many features in common that they may be grouped under the same title, no matter what their situation in the alimentary canal. Thus it is very probable that their point of departure and their causes are the same everywhere. In this paper, I shall consider only the appendicular carcinoids, basing their description on 50 specimens which I have studied personally.

Appendicular carcinoids may form in the body of the appendix where, in growing, they may cause a stenosis of the lumen with retention, followed by acute inflammation. More often, in 46 of the 50 specimens in my collection, they are found at the distal end. If the carcinoid is small, the tip of the appendix is not deformed; if large, it is swollen like a pendulum. This swelling is often the only sign of a possible appendicular tumor but sometimes the presence of tumor is indicated by an opaque, yellowish infiltration of the peritoneal connective tissue and fatty lobules that cover the tip of the appendix.

Section of the appendix shows that the lumen of the tip has disappeared and that it has been replaced by compact tissue, more or less fibrous, hard, yellowish or brownish. To the naked eye the muscular layers seem to be almost intact and always recognizable, even when distended by the tumor and even when the tumor has infiltrated them before involving the peritoneum.

Where the tumor is situated there is no longer an appendicular lumen. The lumen may appear immediately above the tumor; here we may believe that the tumor has caused the obliteration. Sometimes the tumor is separated from the lumen by a variable space where the naked eye perceives nothing but fibrous tissue and

* For the literature, consult the excellent monograph of Wiley D. Forbus, *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 130.

where subsequent microscopic examination reveals no carcinoid cells. This observation is of the first importance for it shows that carcinoids may form in a long stenosed region of the appendix, long deprived of its mucosa and consequently of an epithelial lining.

Histology of Carcinoids: From their structure carcinoids were at first thought to be carcinomata; in fact, they are constituted by columns and masses of epithelia which infiltrate. Their usual benignity and their small size earned for them the name little carcinomata (Lubarsch), then carcinoid (Oberndorfer), under which name they are commonly described. We shall take as a type the carcinoids which form at the tip of the appendix.

When the carcinoids are small, the epithelial columns are situated solely in the axial connective tissue of the appendix; in other specimens, while situated in the axial connective tissue, they invade also the interstices of the circular and longitudinal muscle coats and the nerves of Auerbach's plexus without destroying either the contractile fibers or the nerves. In still other more advanced specimens, they occupy the axis of the appendix, the interstices of the muscle and invade the peritoneal fat. It is obvious that their point of departure is always the axis of the appendix * and that they infiltrate the muscularis and the serosa later. Their development is centrifugal.

Carcinoids consist of epithelial cells grouped in columns of varying breadth and a stroma. We shall study these elements successively in preparations made by the usual methods. The cells are small and of various forms which can be arranged in three groups: round cells, which may be polygonal from reciprocal pressure; palisade cells; and columnar or, better, prismatic cells. Mitoses are rare but not amitotic figures.

Round or polygonal cells are much the most common, forming columns of varying breadth. Those next to the connective tissue are cuboid. The nuclei are central, round and turgid, the network finely reticulated and dotted with minute chromatic granules. In well fixed preparations, the protoplasm is clearly bounded by a

* In a paper which appeared in 1914 and which has only recently come to my attention, Ehrlich supposes that carcinoids arise from Auerbach's plexus. On this supposition he bases his statement that these tumors are immature sympathetic neurocytomata. We shall see why this interesting view is inadmissible. For the moment, we note that it rests on insufficient evidence, for Ehrlich saw only advanced carcinoids which had already infiltrated the muscularis and the plexus myentericus.

very delicate membrane. In some cells the protoplasm is homogeneous and frankly acidophil, in others it is abundant and dotted with acidophil granules of extreme delicacy and great regularity. Most of the cells contain tiny vacuoles, sometimes few, sometimes very many and of uniform size like spongiocytes of the adrenal cortex or xanthoma cells (Fig. 29).

Palisade cells (Figs. 28, 30) are less constant. Usually they collaborate with the round cells in forming the columns, ranging themselves along the connective tissue as on a basement membrane. The nuclei present the same texture as those of the round cells; they are turgid, without puckering, and oval in shape. The nucleus is at some distance from the foot of the cell and all this infranuclear region of the cell is filled with acidophil granules, among which may sometimes be distinguished a tiny diplosome surrounded by a non-granular area. This basal and granular region of the cell has no vacuoles but the vacuoles appear about the nucleus and are often very abundant in the supranuclear region.

Sometimes the palisade cells undergo a sort of stratification; some are prismatic, others remain attached to the connective tissue by a long, slender, granular foot while the nuclear and swollen part of the cell insinuates itself deep among the polygonal epithelia. The cell has the form of a tennis racket, only the broad part containing the vacuoles (Fig. 30). Other cells, attached to the connective tissue by a slender foot, are pointed at the other end also, becoming fusiform.

The columns may consist of palisade cells only, curving inward and resembling remarkably the columns of the glomerular layer of the adrenal cortex of certain animals, the horse, for example.

The columnar or prismatic cells are always grouped around a small round cavity or vesicle, forming a rosette (Figs. 28, 31). The cavity may contain a tiny albuminous droplet, more or less colorable, or a homogeneous droplet of colloid aspect. The apical pole of the cell bounds the cavity; this pole is narrow, always bordered by Kitt-leisten, and consists of a membrane which may be very thin or may be thickened as a striated cuticle. The basal pole rests on the connective tissue or on the polygonal cells. Laterally, the cells are separated by delicate smooth membranes, well outlined. The oval nucleus, similar to those of the palisade cells, occupies the middle of the cell.

The apical pole of each cell is clear, without granules; a tiny diplosome may be detected near the tip of the cell. In some specimens all this supranuclear region is vacuolated. The basal pole is always loaded with granules and is without vacuoles.

These cell forms are connected by all possible intermediary forms and they are often associated in the same column. It is obvious that they do not represent distinct species but rather forms of differentiation of one and the same strain, characterized by nuclei of special structure and by protoplasm which is fundamentally clear and only slightly colorable, in which are often found very tiny acidophil granules and vacuoles, at times numerous but always small and irregular. Moreover, these inclusions which are diffused through the polygonal cells are clearly polarized in the palisade cells. The granules accumulate especially in the basal or connective tissue pole, the vacuoles in the opposite pole. The columnar cells are still more clearly oriented, for the basal pole alone contains granules while the apical pole never has them. Thus the palisade cells and the columnar cells have a granular basal or connective tissue pole and a homogeneous vacuolated apical pole. This orientation is particularly striking in certain specimens where massive columns are penetrated by fine blood capillaries; each of the capillaries is surrounded by a radiating rosette formed by the granular bases of the cells implanted on it.

Beyond the vascular areas just described, the stroma of carcinoids is fibrous or fibro-hyalin, very rich in delicate elastic fibers. There are found also arterioles and venules with muscular walls which scarcely exist in the stroma of carcinomata.

Finally, in that portion of the tumor which develops centrally, inside of the muscular wall of the appendix, smooth muscle fibers are often found among the connective tissue and elastic fibers of the stroma. These may appear in such quantity that they alone form almost all of the stroma, to such a degree that the tumor may be called an adenomyoma or myocarcinoid. Highly important is the fact that this myomatosis does not arise from hyperplasia of the longitudinal or circular layers of the muscle coat; there is always to be found a purely fibrous layer derived from the submucosa which isolates it from the muscularis.

The myomatosis has but three possible sources, hyperplasia of the muscle coat of the arteries or veins, production of muscle fibers

by the connective tissue cells of the stroma, or hyperplasia of the muscularis mucosae. I am inclined to accept this last hypothesis; for the myomatosis is found only in the axis of the appendix, the situation of the fibers of the muscularis mucosae, and not in the longitudinal or circular layers of the muscle coat or in the peritoneal fatty connective tissue when this is invaded by the carcinoid epithelia. In short, the carcinoids seem to have an elective action on the proliferation of the muscularis mucosae.

To sum up their histology, carcinoids resemble ordinary carcinomata of the intestine in the cylindrical form of certain cells; they differ by vacuolation and by the fine granulation of their protoplasm, the granules often accumulating in the vascular pole of the cell.

The Nature of Carcinoids: In 1912, Saltykow, struck by this vascular arrangement of the granules, by their acidophilia and by the endocrine structure of carcinoids, believed them to be tumors arising from aberrant islands of Langerhans. This explanation should never even have been considered for as early as 1907 Oberndorfer had demonstrated that the granules of carcinoids are chromaffin and that their vacuoles are filled with doubly refracting lipoids. Now, the fine granules in the cells of the islands of Langerhans are not chromaffin and these cells never contain doubly refracting fats.

In 1910, Huebschmann advanced the hypothesis that the carcinoids arise from the cells of Paneth or, more probably, from the granular cells discovered in the intestinal epithelium by Nicolas, rediscovered by Kulchitzky, studied later by Schmidt (yellow cells), then by Ciaccio (enterochromaffin cells); but he went no further.

Specific Vacuoles and Granules: On beginning my own studies of carcinoids I found the vacuoles and the granules. In frozen sections the vacuole is seen to be filled by a droplet which stains bright red with scarlet red and with Sudan. The droplets themselves are not doubly refractive but contain crystals which look bright with crossed Nicol prisms. It is probable then that the droplets consist of a mixture of neutral fats and cholesterin esters. Ciaccio's method shows that the walls of the vacuoles contain a very small quantity of lecithin.

From this point of view the fatty inclusions of carcinoids recall those of the cells of the adrenal cortex. On the other hand, the granules are chromaffin and stain black with iron hematoxylin like those of the adrenal medulla. Thus, by the contents of their vacuoles

and by the chromaffin and siderophil reactions of their granules, the carcinoid cells resemble both the cells of the adrenal cortex and those of the medulla and present the features of both in one and the same cell. Now, it is acknowledged that from an embryological, an anatomical, and a functional point of view the adrenal medulla and cortex, the one of sympathetic origin, the other of coelomic origin, are absolutely distinct. Moreover, neither the adrenal nor its tumors ever present prismatic cells grouped in rosettes around a cavity filled with colloid. Therefore, the carcinoids cannot arise from adrenal inclusions.

Next resorting to a new technique which I had already used in the study of melanin, sections of material fixed in the picro-formol of Bouin were immersed in Fontana's ammoniacal silver nitrate. I found that the granules of the carcinoid cells stained dark brown and consequently possessed argentaffin and silver-reducing properties. Sections of adrenal medulla treated in the same way remained colorless, which observation completed the proof that there is no possible identity between carcinoids and the adrenal paraganglion. But this technique applied to sections of the intestine brought out with great clearness the granules of the cells of Kulchitzky.

Thus it became almost certain that the carcinoids arose from a pure proliferation of the enterochromaffin, argentaffin and silver-reducing cells. Since the structure of these tumors resembles that of certain endocrine glands (Saltykow), I called them endocrine tumors and suggested that the chromo-argentaffin cells of the intestine constitute a diffuse endocrine gland.

Since my studies of 1914, a number of researches have been made on carcinoids and on the chromo-argentaffin cells of the intestine. Concerning carcinoids, the opinions may be divided into two groups according to whether or not the writers were familiar with my publications. Thus Abrikossow, following Aschoff, believes them to be nevi of the intestinal mucosa. Krompecher holds them to be basal epitheliomata. Schober groups them among the "progonoblastomata" of Mathias. Engel and Lauche attribute them to embryonic inclusions. Ehrlich makes of them "immature neurocytomata" of the sympathetic.

Those writers who have been willing to adopt my silver technique or any method in which formol fixation is followed by immersion in an ammoniacal silver salt (Delbet and Herrenschildt, Danisch,

Hasegawa, J. F. Martin, W. D. Forbus, Sprafke), have agreed with me that these tumors arise from the chromo-argentaffin cells of the intestinal epithelium.

As for their endocrine nature and the endocrine function of the cells of Nicolas-Kulchitzky, most writers have refused to follow me. In repeating my experiments on the cells of Kulchitzky, histologists and pathologists have confirmed most of the observations made by my forerunners and by myself; but they have interpreted them in a different manner. Let us study the cells of Kulchitzky as they may be observed in the lining of the alimentary canal.

II. THE CELLS OF NICOLAS-KULCHITZKY

Yellow cells (Schmidt); enterochromaffin cells (Ciaccio); argentaffin or silver-reducing cells (Masson); chromo-argentaffin cells (Cordier): The cell of Nicolas-Kulchitzky is characterized chiefly by its granules and secondarily by a special form and structure; for, under certain circumstances and perhaps in certain functional states, the granules may be absent. Post-mortem changes destroy the granules more or less completely, hence the necessity of using strictly fresh material.

The fixative should always contain formol but no alcohol (Masson, old but unpublished studies; Hamperl, Cordier). Formol itself fixes the granules well but fixes the tissues very badly.* The best fixatives are Bouin's picro-aceto-formol and the bichromate-formol mixture. Bichromate gives to the granules a yellowish brown tint; after picro-formol or formol alone, the granules are invisible because of their special refraction but they stain intensely with ammoniacal silver nitrate, with iron hematoxylin and with acid dyes, such as eosin, acid fuchsin and ponceau de xyloidine, as will be described more fully in the section on Technique.

The alimentary canal of all vertebrates contains granular cells similar to the cells of Nicolas-Kulchitzky. In mammals, they are found from the cardia to the anus (Masson, unpublished studies; Hamperl, Cordier), the form varying somewhat with the species and the region. In the gastric glands and in Brunner's glands, these cells insert slender prolongations between the bases of the gland cells; in the human intestine, and consequently in the appendix, their characteristics are as follows (Fig. 1).

* I am perhaps alone in this opinion but the greater my experience the more I am convinced of its truth.

They are scattered singly among the cylindrical cells of the intestinal epithelium, from five to ten in each gland of Lieberkühn, where they are most numerous. They are less frequent in the upper part of the tubule but are found as far as the tip of the villi, where they may be seen desquamated like the other cells of the intestinal mucosa. It is probable that those deep in the tubule are fixed and that only those placed more superficially migrate with their neighbor cells and disappear like them.

In form, the cells of Nicolas-Kulchitzky are almost always conical, with a broad base in contact with the glandular basement membrane, and with a narrow apex bounded by a delicate membrane which is surrounded by the apical membranes of adjacent cells (cells of Paneth, cylindrical cells, caliciform cells). Some of them border the gland lumen, the bulk of the cell being buried among the other epithelia.

The nucleus is round or, more often, oval, turgid and regular in outline except that in some cells the basal pole is hollowed like a cup. The nucleus is never in contact with the basement membrane but rather in the middle of the cell. The nucleus is clear; it contains one or two karyosomes and a network finely dotted with chromatin.

The protoplasm is clear and homogeneous, staining less deeply than the other epithelial cells. There is a Golgi apparatus in the supranuclear region (Cordier) and another in the basal region (Kull). The basal region almost always contains tiny granules which are acidophil, chromaffin, stain with Heidenhain's iron hematoxylin, reduce silver and turn greenish with basic blues. The granules vary in quantity; sometimes they are all beneath the nucleus, sometimes beneath and at each side of the nucleus. Rarely a few are found above the nucleus but never outside of the cell in the intestinal lumen (Cordier). They seem to disappear at certain functional stages of the cell (Cordier).

The granules present a different appearance according to the technique employed. If blocks of tissue fixed in bichromate are treated with silver before embedding (see Technique), the granules are comparatively large, all of the same size and equally black. In paraffin sections treated with silver the granules vary greatly; some of them reduce silver strongly or stain intensely with iron hematoxylin and among these granules some are large and others barely visible; other granules stain neither with silver nor with hematoxy-

lin but with acid dyes. This variable staining may be seen in one and the same cell and also in different cells of the same gland of Lieberkühn, some cells containing more black granules, some more acidophil granules.

In my opinion, these differences in size and staining in tissue treated with silver before or after embedding, depend on the dissolving out by the toluol of some substance which at a certain stage forms part of the granule. Of the nature of this substance, many guesses might be made. Doubtless these variations correspond to different stages of evolution of the granules. From this standpoint it is well to note them but more than this it is impossible to say.

Besides the granules, the basal protoplasm may contain one or two vacuoles, the contents of which stain with Sudan (Danisch).

Thus, in every feature, the cells of Nicolas-Kulchitzky are identical with the cylindrical and argentaffin cells which group themselves as vesicles in the carcinoids. The palisade, fusiform and spongy cells of these tumors are not found in the normal epithelium; doubtless they represent a morphological deviation which depends on their peculiar situation in the interstices of the connective tissue.

Origin of the Normal Argentaffin Cells: My embryological studies, confirmed by those of Parat, have shown me that the argentaffin cells appear in the intestinal epithelium about the fourth month of fetal life in man; they seem to spring directly from the cells of the entoderm. Kull, however, studying them in the chick embryo by a mitochondrial method, not by ammoniacal silver, derives them from mesenchyme cells that have invaded the epithelium. This opinion cannot be maintained; the very figures published by Kull forbid it. There is not the slightest possible resemblance between the mitochondria of the connective tissue cells of the mucosa and the chromaffin granules of the chromo-argentaffin cells, although these latter also stain with Altmann's acid fuchsin. Their dimensions are very different.

Influenced by my own work (see Argentaffin-cell Neuroma, page 192), Danisch seeks to show that the chromo-argentaffin cells arise from the solar plexus and migrate to the intestinal epithelium in the fourth month of fetal life. His figures are not very convincing and, according to his own statement, represent macerated tissue. Moreover, Danisch uses Agdhur's silver technique which indeed colors the argentaffin cells but not specifically and colors also certain

chromaffin cells of the sympathetic paraganglia. His conclusions should be rejected.

There are two pathological observations which tend to show that the chromo-argentaaffin cells are really of entodermic origin:

1. In chronic gastritis, they are found in abundance in those glands of intestinal type which form in regeneration of the mucosa.

2. They are sometimes found (Hamperl, Martin and Masson) in cancers of the intestinal tract and in their metastases, mixed with ordinary cylindrical cells, and may be traced easily to their origin in the common type of cylindrical cell.

Function of the Argentaaffin Cells: The granules of the cells of Nicolas-Kulchitzky have inspired many physiological hypotheses. Ciaccio holds that, because of their chromaffinity, the cells produce adrenalin which is poured into the intestinal canal. However, the specific reaction to ammoniacal silver indicates that the substance secreted differs from adrenalin. The chromaffinity and the silver reduction are proofs of reducing power; more than this cannot be said at present.

The situation of the granules at the base of the cell indicates an endotropic polarization of the cell; for this reason I have advanced the idea that they have an endocrine function. In their normal state this function is doubtless not exclusive. Kull states that the cells have two Golgi apparatuses, one apical, the other basal. According to Cowdry, the situation of the Golgi apparatus is connected with the pole of discharge of the cell. Moreover, in the carcinoids, the presence of cavities of secretion bordered by cylindrical cells and filled with an albuminous or colloid liquid, demonstrates that these cells secrete something from their apical pole; in a word, they are exocrine.

However, this does not exclude a concomitant endocrine function. Liver cells normally exhibit two polarities, external and internal. The pancreatic cell is capable of exhibiting them successively and, to return to the carcinoids, it is obvious that if the palisade and the polygonal cells of the larger masses secrete anything, either they retain it in their interior or eliminate it into the vessels. If then the cylindrical argentaaffin cells of the normal intestine and of the carcinoids secrete in both directions, the cells of the same lineage which do not border the lumen of a tubule have certainly lost their exocrine function and can be only endocrine. This endocrine function is,

perhaps, not without some connection with the proliferation of the muscle fibers which is so often observed in the stroma of carcinoids.

Cordier has expressed doubt of the endocrine function of the cells of Kulchitzky; in asserting their exocrine function, he relies on the disappearance of the granules after injection of pilocarpin. I do not deny this disappearance but it proves nothing against my thesis; for Cordier has been unable to show in which direction, intestinal cavity or interstitial tissue of the mucosa, the product which results from the destruction of the argentaffin granules is excreted.

In sum, our knowledge of the normal chromo-argentaffin cells shows their identity with the cells which constitute carcinoids; but their physiological rôle remains obscure and their endocrine function in particular remains to be proved.

III. ARGENTAFFIN-CELL NEUROMA

(A) ARGENTAFFIN-CELL NEUROMA IN OBLITERATED APPENDICES:

If carcinoids are really endocrine tumors composed exclusively of enterochromaffin and silver-reducing cells, there is reason to believe that the normal enterochromaffin cells, when situated in the intestinal epithelium, possess similar properties and functions, and that these are endocrine. How shall we verify this?

There comes to mind Laguesse's famous experiment in which he demonstrated the endocrine function of the islands of Langerhans by ligating the external pancreatic ducts. The exocrine acini atrophied rapidly; the islands persisted. At one stroke, he demonstrated their purely endocrine function and their rôle in the metabolism of glucose by the elaboration of a substance which has since received the name insulin. Unfortunately, an experiment of this kind cannot be carried out on the intestine, especially the appendix, ligature of which would be followed immediately by subacute inflammatory symptoms. At this point, I thought of utilizing the experiments performed by nature and examined appendices in which the lumen had been obliterated by cicatricial stenoses, or such as were so considered by most writers, in the hope of finding argentaffin cells in that axial connective tissue which replaces the former lumen and the mucosa.

If the accidents of cicatrization had incarcerated fragments of the glands of Lieberkühn and isolated them from the intestinal cavity,

the argentaffin cells, if really endocrine, might survive the others and persist in the connective tissue just as the islands of Langerhans persist after disappearance of the acini.

My first studies in this direction did not confirm this anticipation but they enabled me to draw three unexpected conclusions:

1. In the axial connective tissue of obliterated appendices there is always to be found a discontinuous, more or less prominent, muscular sheath formed by the persistence of the smooth muscle fibers of the muscularis mucosae.

2. Inside of this sheath there are always nerves, non-medullated, always large and often clustered together to form neuromata, included in connective tissue which is sometimes fibrous, sometimes edematous, sometimes hollowed out by an axial lacuna.

At first, I believed that these neuromata, so frequent in obliterated appendices, were amputation neuromata similar to those of the cerebrospinal nerves and that they were caused by division of sympathetic filaments of the mucosa by an ulcerative process. About this time, Maresch made similar observations and within a few days of each other we published almost identical papers on appendicular neuromata considered as amputation neuromata.

3. At the time when my work appeared, this explanation did not satisfy me altogether. In fact, in pursuing my studies, I had ascertained that the nerves which persisted in the appendicular axis and especially in the neuromata always contained cells, the protoplasm of which was dotted with argentaffin granules. Thus the long-sought cells were found again but this time inside of the nerves and never outside of them in the interstitial connective tissue.

This observation, the constancy of which I was able to verify, seemed to me to be of fundamental importance. It no longer permitted the interpretation of the appendicular neuromata as common amputation neuromata, for these do not contain argentaffin cells. From another point of view, it might be asked if carcinoids and argentaffin-cell neuromata were not related and even if they had not a common anatomical basis.

The Neuromata: The neuromata are composed of compound and plexiform non-medullated fibers identical with those of the intestinal mucosa, but voluminous, rolled up in masses and in close apposition. The trichrome stain colors their collagen sheath blue, the neuroglia bright red. The neuroglia is formed of ramifying and

anastomosing tubes, the contents of which, consisting doubtless of neurites, stain pale pink (Figs. 21, 22, 23, 24, 25).

The neuromata vary greatly in size. Some are microscopic, some large enough to be visible to the naked eye. They may attain a diameter of from two to three millimeters without piercing the sheath formed by the vestiges of the muscularis mucosae. Only twice have I seen neuromata which had passed this usual boundary and broken through not only the muscularis mucosae but the external muscle coat as well.

The neuromata are usually numerous; the smaller the tumors, the more easily counted. I have counted from 50 to 60 in one centimeter of appendix! When large, they tend to fuse in masses or the nerve fibers interlace in an inextricable tangle. It is important to note that they are always connected with one another by plexiform fibers, some transverse and oblique (Fig. 23), linking neuromata which have formed at the same level, others longitudinal (Figs. 23, 25), connecting tumors at different levels. In some well-oriented, longitudinal sections the appearance of these neuromata linked together recalls the nerve chain of an arthropod or worm.

Besides these interconnections the neuromata anastomose laterally and externally with Meissner's plexus of the submucosa which, however, exhibits no increase of ganglion cells or fibers.

The appearance of the neuromata varies in different specimens. Some are formed of slender fibers, rich in Remak's nuclei; these are evidently in active growth. Others consist only of large fibers with scanty nuclei; these seem to have completed their growth (Fig. 22). Others again are invaded by lymphocytes; the fibers are widely separated, shrunken and clearly in full retrogression (Fig. 21).

It is probable that the life of these neuromata is ephemeral and that they disappear one after another more or less completely; but this solitary retrogression and lymphoid infiltration would occur only if each of them had a certain autonomy in spite of its connections with its companions and with Meissner's plexus. Almost every obliterated appendix examined contained at least vestiges in the form of longitudinal and axial nerve fibers.

Are these structures really nerves and neuromata? If the answer is sought in Cajal's or Bielschowsky's technique of silver impregnation and reduction, these are not neuromata; for silver never colors neurites in them. All my attempts in this direction have failed,

as well as those of Schweizer and other workers. Schweizer concluded that these supposed neuromata are nothing but neurinomata (Schwannomata); others who, disregarding my warning, have used only the silver-reduction methods in studying obliterated appendices, have seen no nerves whatever and have simply denied my statements (Lauche).

Silver impregnation is the most capricious of all histological methods. On occasion, it colors neurites, neuroglia, elastic fibers, reticulum, collagen, we know not how or why. The many modifications of silver technique suffice to show its unreliability. As for coloring neurites, outside of the central nervous system the results are so inconstant and imperfect that I refuse to accept them as characteristic. When silver reveals black fibers in the nerves, I believe indeed that they are neurites; if silver shows nothing in a tissue which other methods, the trichrome stain especially, stamp as nervous, I reject the negative evidence. Moreover, as we shall see later, if the mucous membrane of the normal appendix is treated by Cajal's or by Bielschowsky's method, very few neurites take the stain, although the trichrome stain reveals an abundance of non-medullated fibers. It must be conceded then, not that there are no nerves in the intestinal mucosa, but that the greater number of nerves in the intestinal mucosa escape silver impregnation; now, by their topography, as we shall see, the neuromata of the obliterated appendix belong to this plexus of the intestinal mucosa.

There is another argument against the neurinoma hypothesis. All neurinomata with which we are familiar arise from localized proliferation of the Schwann cells; they contain no argentaffin cells; they are independent of one another; they do not regress. The nerve tumors under consideration communicate by longitudinal fibers, uniting one with another, and they regress individually as if, in spite of their continuity, they enjoy a certain autonomy. Hence the hypothesis that they are true neuromata, just as the nerves of the mucosa are true nerves notwithstanding the absence of argento-phil neurites; and that their trophic center is neither Meissner's nor Auerbach's plexus, neither of which participates in their growth or in their degeneration.

Argentaffin cells: Argentaffin cells are always present in the growing or fully developed axial neuromata of the obliterated appendix, either singly or in groups of from two to twenty cells of the most

varied forms. Some are polygonal from reciprocal pressure, or more or less rounded.

They are enclosed in a neuroglial syncytium but their external limits cannot be determined exactly. They seem to form a part of the syncytium. Their nuclei are similar to those of carcinoid cells. Their cytoplasm is loaded with silver-reducing chromaffin, siderophil and acidophil granules, and often hollowed out by vacuoles containing lipoids. The droplets escape from the cell bodies like a product of secretion and disappear in the neuroglia. To this peculiar process of intranervous internal secretion I have given the name *neurocrinia*. Cells which belong to this type I call *neurocrine cells* (Fig. 11).

Other cells have no distinct cytoplasmic body; their nuclei are enclosed directly in the neuroglial syncytium and are surrounded by a few silver-reducing granules. These argentaffin cells are no longer individualized, they form an integral part of the Schwannian syncytium (Figs. 9, 10).

Other cells have the clear contour of ganglion cells, the nucleus at times vesicular, poorly chromophil and provided with rounded nucleoli. Only their silver-reducing granules identify them as argentaffin cells. Basic blues may show angular figures resembling Nissl bodies. I call them *cells of ganglion type* (Fig. 10).

Still other cylindrical or cuboid cells are arranged in a rosette around a cavity filled with a substance of colloid appearance, the whole being enclosed in the neuroglial syncytium. Only their basal region is filled with the silver-reducing granules. These cells correspond to the cells of Kulchitzky; they are *cells of the intestinal type* (Figs. 9, 10).

In short, in these neuromata we find cylindrical argentaffin cells and lipo-secreting cells as in carcinoids; but besides these we see two other types, which belong to the same strain however, argentaffin cells incorporated in the Schwannian neuroglia and argentaffin cells of ganglionic aspect. All these cells are in intimate contact with the neuroglial cytoplasm and completely separated from the interstitial connective tissue.

There is still more. If we study serial sections of neuromata in regression infiltrated with lymphocytes we never find argentaffin cells of any kind; but they are always to be found in the other neuromata. This observation together with the obvious indifference of

the ganglion cells and nerves of Meissner's and Auerbach's plexuses to the growth and degeneration of the axial neuromata of the appendix, leads me to believe that these neuromata depend exclusively on the multiform argentaffin cells contained in their fibers.

Without my former studies on carcinoids and the cells of Kulchitzky, I should have believed these neuromata to be ganglioneuromata of the sympathetic. I should have accepted the polygonal cells as paraganglionic and the cells of the ganglion type as sympathetic. As for the rosettes, I should have held them to be neuro-epithelial. Unfortunately, this latter interpretation could not have been advanced without embarrassment; for the embryonic sympathetic contains no hollow rosettes with colloid contents, bordered by cells with a striated cuticle but rather groups of sympathogonia without central cavities. The rosettes, the silver-reducing granules and the colloido-secretory cells give to the neuromatous complex an especial character which is not seen in cerebrospinal ganglioneuromata or in sympathetic ganglioneuromata. On the contrary, they are too like the normal cells of Kulchitzky and the carcinoid cells to neglect to inquire into their possible intestinal origin.

(B) NEUROMATOSIS OF THE PERMEABLE APPENDIX: *Origin of the neuromata of obliterating appendicitis*: It was at this point that I took up the study of appendices still provided with a mucosa. It was obvious that if argentaffin cells occur in the neuromata, they had migrated into the nerves while the epithelium of which they normally form a part still existed. I began by examining healthy appendices or those supposed to be healthy, without pathological history, excised during laparotomies performed for various reasons, and then realized the peculiar richness of the periglandular nerve plexus of the appendix, as shown by the trichrome stain. This richness is maximum between the ages of 18 and 35 years and is far greater than in any other part of the intestine. The nerves of this plexus ramify in direct contact with the gland tubules deep in the reticulated tissue of the mucosa.

I made this observation also, that ammoniacal silver nitrate nearly always reveals an occasional argentaffin cell in these nerves. Their detection may require prolonged search for there may be only one cell in thirty or forty serial sections. As already stated, I applied the methods of Cajal and of Bielschowsky to these nerves without success.

Turning to appendices with pathological history but extirpated in the absence of any acute inflammatory crisis, I found almost always an abnormal number of silver-reducing cells in the nerves of the periglandular plexus (Fig. 13), and exceptionally a few in the inner layer of Meissner's plexus. These cells may be very few and found only after diligent search, there being only one or two in four or five sections of 5 microns; in other specimens, they are innumerable, either scattered or massed together, always inside of the nerve filaments and never in the interstices of the lymphoid tissue (Fig. 2).

In the periglandular nerves, these cells have the same characteristics as those already described in the neuromata; they may be neurocrine, Schwannian, ganglionic or cylindrical. These latter are not constant, the neurocrine cells always predominating.

Finally, the nerves inhabited by the cells are broadened, hypertrophied and increased in number. In some specimens they form actual neuromata, diffuse or localized, which push the muscularis mucosae before them (Figs. 16, 17). Their fibers are closely approximated, narrowing the meshes of the reticulum. Such a neuromatous mucosa has a characteristic clearness owing to a diminution in the number of lymphoid cells.

The neuromata are often multiple. They are situated solely beneath the gland tubules, never in the lymphoid follicles. They are connected with Meissner's plexus by non-hypertrophied fibers which traverse the muscularis mucosae. In these neuromata a certain number of neurites may be impregnated with silver, especially about their connections with Meissner's plexus, but many of them take the silver no more readily than do the neurites of the normal gland plexus or of the neuromata of the obliterated appendix.

A step further, in studying *incompletely obliterated appendices*, I found that their neuromata were continuous with the plexus of the mucosa and that they represent an extension of the argentaffin-cell neuromata which had developed in the still permeable portion of the mucosa.

Thus the neuromata of obliterating appendicitis arise from the hyperplastic plexus of the mucosa, this plexus being inhabited by argentaffin cells. If the plexus contains the argentaffin cells, it will survive and undergo hyperplasia even when the epithelial lining of the appendix has disappeared.

Origin of the Intranervous Argentaffin Cells: It remained to dis-

cover the source of the intranervous argentaffin cells. The first thing that struck me was that no matter how great their abundance, I had never seen one in mitotic or amitotic division. Whence could they come if not from the epithelial lining? Minute examination showed me that it was indeed thus and that all the argentaffin cells enclosed in the nerves result from intranervous budding from the glands of Lieberkühn. The budding epithelia then separate from their gland matrix, migrate into the nerves of the mucosa and differentiate into the various types already described.

Budding: To demonstrate argentaffin cells in the nerves of the plexus of the mucosa is an easy task if the observer is willing to use my technique, ammoniacal silver nitrate and the trichrome stain. To trace their migration from the Lieberkühnian lining into the nerves is much more difficult and rarely possible. This will be readily understood on reviewing the conditions that must happen to coincide so that a process of such short duration and of such capricious orientation may occur at the precise moment of tissue fixation, in sufficient abundance to exhibit the various stages and in a direction that coincides with the plane of the sections. I should add that the process is momentary, that it occurs under circumstances beyond our control, usually during appendicular colic with slight inflammation, and in subjects from 18 to 25 years of age. Of the 1200 appendices examined, I found budding in only fifteen. To give an idea of the difficulty of this work, I may say that in those specimens in which the buds were most numerous, I counted on an average only one in seventeen serial sections of 5 microns thickness. That is to say, finding the buds is a matter of perseverance.

I trust that I may be pardoned for giving these details. The object is not to discourage those who may wish to control my observations but to warn them against the skepticism which might follow researches insufficiently pursued.

Figures 3, 4, 5, 6, 7, 8, 15, make unnecessary a long description of this process. Budding begins with an increase in the number of nuclei in a limited area of the lining of a gland of Lieberkühn. The multiplication of nuclei is always amitotic. It is strictly localized, either at the tip of the gland tubule or more often a little to one side. It is never seen in the annular zone where mitotic figures are found or above this point.

The nuclei invariably belong to the *indifferent cells* and in the

beginning preserve their irregular contours and customary flabby aspect. Their multiplication obliges them to arrange themselves in several layers, some in the middle zone of the cell, some close to the basement membrane. Granules are never seen in the cytoplasm of these cells the limits of which are so indistinct as to give to them as a whole a syncytial aspect.

The basal ends of two or three adjoining cells now elongate, uniting to form a small projection which first pushes before it and then perforates the basement membrane and at last finds itself directly in contact with a nerve filament of the periglandular plexus (Figs. 3, 4).

Several nuclei now migrate into this pseudopod which elongates and buries itself in the nerve. The epithelial bud thus formed may remain small, consisting of few cells, or on the contrary it may grow large and multicellular. In the latter instance it assumes a spherical form and remains for a time attached to the gland by a slender pedicle. It is then and then only that the appearance of the cell changes. The nucleus swells, the chromatin becomes regularly and finely reticulated. Silver-reducing granules appear abruptly in the cytoplasm. These changes are observed first in the cells which have pushed farthest into the nerve, the first to emigrate, then in the others (Figs. 5, 6, 7, 8).

Soon the pedicle of the bud shrinks, parts, and the cells find themselves cut off from the original gland tubule. In some lucky sections in which the tissue happened to be fixed shortly after the division of the pedicle and where the invaded nerve has been cut longitudinally, a group of argentaffin cells may be seen isolated in the nerve a few hundredths of a millimeter from the lining of the gland tubule. This nerve is in direct contact with the Lieberkühnian cells which remain in the tubule and these cells insinuate slender prolongations which blend with the neuroglia (Fig. 15). Intestinal cells and neuroglial syncytium are continuous. Such pictures have a further value; they show that the various views of budding represent the emigration of epithelial cells into the nerves and not the immigration of formerly intranervous cells into the gland tubule.

Migration: After their separation from the intestinal epithelium, the cells which have become argentaffin may remain in the vicinity of the parent gland. More often they continue their migration inside of the filaments of the subglandular plexus and by this path

reach a deeper level of the mucosa. Rarely do they reach the muscularis mucosae or the superficial branches of the submucous plexus, never beyond this point.

Migration is preceded by dislocation of the cells of the bud which, when liberated, seem to insinuate themselves between the nerve fibers. Their advance is checked here and there at a crotch of the plexus by the interlacing nerve fibers. The ganglion cells (Fig. 14), present a particularly insurmountable obstacle. When budding is very active and frequent, the nerves beneath the budding gland may be seen to be invaded by argentaffin cells which, having been separated for a time, now range themselves in rows or pile up in masses as if all of them had been arrested by the same obstacle. It is in these masses resulting from blocking of the cells at certain points, and not by multiplication that the cells adapt themselves to one another and form vesicles or rosettes with colloid contents (Figs. 8, 9, 10, 12).

Differentiation of the argentaffin cells: In recent cell groups we may observe differentiation into one or the other type, intestinal, neurocrine, ganglionic or neuroglial (Figs. 9, 10). This phase of differentiation of the intranervous argentaffin cells is short, apparently as short as the phase of glandular budding which preceded it. It is seen clearly only in rare instances where budding has been met in great activity. Budding terminated, each emigrant cell soon assumes its type. The submucous plexus is crowded with polymorphic argentaffin cells, all isolated from the epithelium and capable of persisting for a long time, doubtless for years. It is in this stable form that we most frequently find the intranervous argentaffin cells in chronic, non-obliterating appendicitis and in the axial neuromata of the obliterated appendix.

Beginnings of nerve hyperplasia: In budding appendices, the periglandular plexus presents great inequalities in the caliber of the nerve fibers, the most voluminous being precisely those which have been invaded by the argentaffin cells. The nerves are enlarged not only at the points occupied by the argentaffin cells (Figs. 7, 8, 9, 10, 11, 12) and especially by the cell masses, but also beyond the cell masses where they are constituted by nerve fibers only. The enlargement of the nerve is due to an increase in the number of its constituent fibers, a multiplication due doubtless to the presence of argentaffin cells. The hyperplasia may remain limited to a few nerves or it may in-

volve a whole region of the periglandular plexus; it may not only enlarge the fibers already existing but also produce new ones which interlace, thicken and end by forming the neuromata already described.

The growth of the nerve fibers is not attended by proliferation of the argentaffin cells which become more widely separated as the neuromata are more voluminous. There is, however, an abundant amitotic multiplication of the neuroglial nuclei, Remak's nuclei, which increase in number in proportion to the increase of the neuromatous mass. The growth remains strictly limited to the intramucous plexus; the submucous and the myenteric plexuses do not participate in any way, save for rare exceptions which I shall describe in another paper.

The emigration period: In my own experience, as already related, gland budding, precursor of nerve hyperplasia, does not take place at all times. All of the subjects on whom I made these observations were between 18 and 25 years of age, the period during which, normally, the periglandular plexus has its maximum growth. Furthermore, all of these appendices presented slight inflammatory lesions involving the mucosa or had given rise to symptoms of slight acute appendicitis several days before intervention. In order to produce abundant budding it appears that two conditions must coincide, a non-mutilating irritation of the mucosa and the active period of normal neurogenesis.

On the other hand, all appendices, even normal ones, may present an occasional argentaffin cell in their nerves and these cells also are of intestinal origin. Therefore, we may suppose that the large number of buddings observed between the years of 18 and 25 both during and following slight inflammatory crises, and represented in the sequel by the presence of argentaffin cells in great numbers in the nerves, represents an exaggeration, under the influence of an irritation occurring at a favorable moment, of a normal process of neurogenesis too discrete to be detected in the healthy appendix. Thus the excessive neurogenesis which prepares the way for neuromatosis seems to be determined by an ordinary and non-mutilating acute appendicitis.

IV. ORIGIN OF CARCINOIDS

During the study of so many appendices, chance has enabled me to observe many carcinoids. As stated in the beginning of this paper, I have examined 50 of these tumors. Many of them were given me by pathologist friends; all of these were large. In my own material I have found 15, some of which were of particular interest because of their small size. Five were invisible to the naked eye; all had developed in neuromata, either terminal neuromata such as are found in obliterated appendices, or lateral neuromata, contiguous to a mucosa still retaining its epithelium.

In all of these specimens I was able to convince myself (Fig. 27) that the carcinoid cell columns had resulted from proliferation of intranervous argentaffin cells of the neurocrine type. These cells, piled up in the nerve fibers, finally rupture their sheaths and infiltrate the interstitial tissue of the neuroma, then that of the submucosa. These neurocrine cells proliferating in the connective tissue assume the characteristic appearance of carcinoid cells. They still secrete fatty droplets but these accumulate in their cytoplasm making it spongy. This results, perhaps, from the impossibility of eliminating their secretion into the nerves, for they are now buried in connective tissue. Proliferating in such an abnormal medium as connective tissue, the neurocrine cells group themselves like ordinary endocrine cells.

SUMMARY AND CONCLUSIONS

1. The axial region of completely obliterated appendices often (86 per cent of the specimens) contains nerves and neuromata enclosed by a discontinuous sheath formed by vestiges of the muscularis mucosae. These neuromata always contain argentaffin cells of divers forms. If the argentaffin cells disappear, the neuromata regress and are absorbed individually, notwithstanding their connections with Meissner's plexus of the submucosa.

2. Study of partially obliterated appendices shows that these neuromata arise from the periglandular plexus and that they often continue the neuromatous evolution which had commenced when the mucosa still contained gland tubules.

3. This periglandular and subglandular neuromatosis always occurs in nerves inhabited by argentaffin cells.

4. The intranervous argentaffin cells spring from the epithelium that lines the bottom of the glands of Lieberkühn.

Reversing the order in which these observations were made suffices to reconstitute the probable and logical chronological order of the phenomena. Certain intestinal cells bud out and migrate into the nerves. Here they become argentaffin. They differentiate into various forms, cylindrical cells grouped in rosettes or vesicles, cells of ganglion type, of Schwannian type, neurocrine cells. The nerves containing them grow and become neuromata, or disappear if the cells themselves disappear.

Carcinoids result from the autonomous proliferation of the isolated neurocrine cells present in the neuromata. In short, by its neuromata and by the argentaffin cells which determine their growth and which are linked with their persistence, the periglandular plexus of the appendix exhibits a remarkable autonomy.

If the cells possessed no specific granules and if we were ignorant of their intestinal origin we would be led to believe that some of them were derived from the sympathetic system, ganglionic or Schwannian, others chromaffin and paraganglionic, and the carcinoids, issue of the latter, would be sympathetic paragangliomata as stated by Danisch. However, their silver-reducing granules, their lipoids and their entodermal origin upset this hypothesis and suggest two others:

Either the nerve plexus is of sympathetic origin and proliferates under the irritating or secretory influence of the cells which have emigrated from the intestinal epithelium (but how then shall we explain the diverse morphology of these cells and especially their invariable migration into the nerves,* never into the lymphoid interstices?); or the periglandular plexus consists of a mixture of fibers, some sympathetic, others belonging to another nervous system, autonomous and autochthonous, of entodermic origin. The fibers of this latter system, like the neuromata which spring from

* Van Campenhout has recently shown that phenomena similar to those that I have seen in the appendix take place in the fetal pancreas of various mammals; they consist in emigration of cells from the primitive endocrine islands into the pancreatic nerves. The emigrated cells constitute the "sympathetico-insular complexes" to which the author freely attributes an endocrine function. Without doubt, he is right; but we might inquire if there is not something more and if certain insular cells do not take part directly in the genesis of part of the pancreatic nervous system.

them, are refractory to silver impregnation and thus their precise origin escapes us.

In the normal state might not this origin be in certain cells which are mingled with the glandular and absorbent cells just as the olfactory fibers spring from certain cylindrical and nevertheless ganglionic cells of the pituitary epithelium? From this point of view we could understand their proliferation and their elective migration into the nerves which are already in continuity with them, their forms of differentiation following exclusion from the epithelium, and the growth of the nerves.

Is it forbidden to consider the possibility of a neurentoderm, an entodermic placode, of which the cells of Kulchitzky are the sole manifestation in normal conditions? In this hypothesis, the neuromata of neurogenic appendicitis on the one hand and the carcinoids (paragangliomata of the neurentoderm?) on the other hand would illustrate the complexity of this nervous system which permeates the entire intestine (the ubiquity of carcinoids proves this) but which is not demonstrable with our present technique.

My hypothesis of the autonomy of a part of the periglandular plexus receives indirect confirmation from the demonstration of a neuromatosis which is undeniably linked with the sympathetic system. These neuromatoses are very different from those described in these pages. I shall reserve their description for a future paper.

TECHNIQUE

Fixation:

Bouin's Fluid

Formol.....	10.
Water.....	30.
Glacial acetic acid (or 10 per cent trichloroacetic acid)	2.
Picric acid.....	to saturation

Optimum fixation time: 3 days.

Do not wash in water. Dehydrate immediately in alcohol and embed in paraffin.

Regaud's Fluid

Formol.....	20.
3 per cent aqueous solution of potassium bichromate.....	80.

Optimum fixation time: 24 hours.

Wash in running water. Embed in paraffin.

Sections: Sections of 5 microns should be numerous and in series. The lesions to be studied are always localized; we must multiply the chances of finding them and be able to study them in three dimensions. The buds and the neuromata cannot be understood without many serial sections.

Affixing the sections: Dissolve 0.05 gm. gelatin (in practice, a bit of ordinary sheet gelatin one-fourth inch square) in 20 cc. distilled water, warming the water over the flame. Filter a few drops of this solution on the slide and float the section on it. Place the preparation on the warm plate at 40° C. As soon as the section spreads, remove the slide, hold the section in place with a brush or needle and stand the slide upright to drain. Blot with absorbent paper and dry in the oven at 40° C in formalin vapor secured by leaving in the oven an open bottle of formalin.

This is the only method which gives perfect adhesion of the sections no matter what the fixative, the duration of the stain, or the temperature employed.

Staining:

The Trichrome Stain

First stage: Staining the nuclei with iron hematoxylin.

The sections, freed from paraffin by toluol, alcohol and water, are immersed in 5 per cent iron alum previously heated to 45 or 50° C for 5 minutes. Wash in water.

Stain for 5 minutes at 45 to 50° C in Regaud's hematoxylin.

Hematoxylin.....	I.
Alcohol 95 per cent	10.
Glycerin.....	10.
Distilled water	80.

Rinse with 95 per cent alcohol.

Differentiate in picric alcohol, which is more selective than iron alum.

Alcohol 95 per cent saturated with picric acid.....	2 parts
Alcohol 95 per cent	1 part

Wash in running water.

Second stage: Staining the cytoplasm and the collagen.

Prepare the following solutions:

(A) Acid fuchsin	0.3
Ponceau de xyloidine*	0.7
Distilled water	100.
Glacial acetic acid	1.
(B) Phosphomolybdic acid	1.
Distilled water	100.
(C) Glacial acetic acid	2.
Distilled water	100.
Aniline blue	to saturation

Stain in *A* for 5 minutes.

Rinse with distilled water.

Differentiate in *B* for 5 minutes.

Without rinsing, pour 10 drops of *C* on the section, tilt the slide a few times for thorough mixing and let stand for 5 minutes.

Rinse in distilled water.

Back to *B* for 5 minutes.

In 1 per cent acetic acid water for 5 minutes.

Dehydrate, clear and mount in salicylic balsam.

Results: Nuclei black; argentaffin granules black or red; cytoplasm and neuroglia vermilion red; collagen intense blue.

SILVERING THE REDUCING GRANULES

(A) *Silvering the sections:*

Fixation: Despite the advice of many writers, tissue that is to be treated with silver should not be fixed in potassium bichromate, which in my opinion gives poor results. Formol preserves the granules very well but the delicate cytoplasmic structures very badly. The picro-acetic formol of Bouin is the fixative of choice.

Prepare the ammoniacal silver nitrate solution as follows:

To 100 cc. of a 20 per cent aqueous solution of silver nitrate, add aqua ammonia drop by drop, shaking well, until the precipitate of silver oxide is just dissolved; then add a few drops of the 20 per cent nitrate until there is a persistent opalescence. The fluid should have no odor of ammonia. Add distilled water to 200 cc., the solution now containing 10 per cent of silver nitrate. Keep in a strictly clean glass bottle and filter just before use.

* Of the brand *Microcolor*, 35 rue Escudier, Boulogne-sur-Seine (Seine), France. For this purpose, other brands of ponceau are very inferior.

The sections, affixed to slides with gelatin, are immersed in toluol, alcohol and then in distilled water, then in the ammoniacal silver nitrate at room temperature in the dark.

In 4 or 5 hours, the argentaffin granules turn yellow, then brown. In 24 hours or in 36 hours at the longest, the silver is completely reduced. Within this time limit, the silver is reduced also on certain coarse granules sometimes contained in the macrophages of the reticulum, granules of lipofuscin or of purin products, all very different from the fine granules of the argentaffin cells. Too long immersion colors the nuclei and the connective tissue.

After immersion in the ammoniacal silver for 24 hours or 36 hours at the longest, the sections are washed in water and toned in the gold bath as follows:

(A) Ammonium sulphocyanide.....	6.
Distilled water.....	100.
(B) Sodium hyposulphite	6.
Distilled water.....	100.
(C) Gold chloride	2.
Distilled water.....	100.

Mix 1 cc. *A* with 1 cc. *B*; add *C* until there is coarse precipitation and pour over the sections. Toning is instantaneous.

Rinse with 6 per cent hypo. Wash in running water.

Result: The argentaffin granules are opaque black.

It is well to stain the background, either with Cajal's picro-indigo-carmin or, better, by the second stage of the trichrome as described above, ponceau-acid fuchsin-phosphomolybdic acid-aniline blue. I recommend the latter especially; for it brings out all the tissue elements perfectly.

(B) *Silvering in the block:*

Fix in Bouin for 3 days.

Cut slices 2 to 3 mm. thick; wash them in running water for 24 hours.

Immerse for 24 hours in a solution of 2 drops aqua ammonia in 100 cc. distilled water.

Immerse for 24 hours in the ammoniacal silver nitrate diluted with 3 volumes of distilled water.

Rinse in distilled water.

Tone in Cajal's mixture for 24 hours:

Ammonium sulphocyanide.....	3.
Sodium hyposulphite.....	3.
Distilled water	100.
Gold chloride (1% sol.).....	1.

Wash in water for several hours, embed in paraffin or celloidin.

All the argentaffin granules are opaque black (see the text); the nuclei are brownish. Counterstaining is unnecessary. It is scarcely necessary to add that the nerves are never impregnated by this method.

SUPPLEMENTARY METHOD

Impregnation of the neurites in appendices fixed in Bouin:

Fix as usual in Bouin for 3 days.

Preserve the tissue in 5 per cent neutral formol for 2 months.

At the end of this time, cut slices from 1 to 2 mm. thick, wash them in pure distilled water for 12 hours and then for another 12 hours in a mixture of 2 drops aqua ammonia in 200 cc. distilled water.

Immerse in the ammoniacal silver so diluted as to contain from 2 to 2½ per cent of silver nitrate and keep for 6 days in the dark.

Reduce for from 6 to 12 hours in:

Formol.....	5.
Pyrogallol	0.5
Water	50.

Embed in paraffin and section.

This method is inconstant and capricious, as are all methods with reduced silver. Its sole advantage and sole indication is that it sometimes brings out clearly the sympathetic neurites of the appendix after picro-formol fixation, which is never accomplished by the technique of Cajal or of Bielschowsky under the same conditions. It does not blacken the neurites (neuroentodermic?) of the mucosa or of the neuromata. It demonstrates the silver-reducing granules but not specifically; for the pyrogallol reduces the silver on the most diverse tissue elements, the nuclei, sometimes the peripheral neuroglia, the various pigments, etc.

This method is of secondary importance; its results should be controlled strictly by my other methods and, in any case, it should

never be trusted alone. I mention it here chiefly to correct an error of Sprafke, to whom I would recommend this method as the method of choice in the study of neurogenic appendicitis.

The photomicrographs are the work of Dr. Charles Simard, to whom I am greatly indebted for his cordial collaboration.

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DESCRIPTION OF PLATES

PLATE 44

FIG. 1. Chromo-argentaffin cells of the normal intestine.

On the right, section of jejunum after silver treatment in the block. Tip of gland of Lieberkühn with two chromo-argentaffin cells, the granules relatively large and opaque, massed in the basal portion of the cell. The granules conceal the sides of the nucleus; a few are found above the nucleus. The reticulated tissue of the jejunal submucosa contains a few delicate nerve filaments, much fewer than the appendicular mucosa, even normally.

On the left, duodenum. Silvering of the sections. Argentaffin cell in the middle of a gland of Lieberkühn. Tiny uniform granules massed in the basal pole of the cell; a few above the nucleus. Note that they are much smaller in silvered sections than when silvered in the block. $\times 1100$.

FIG. 2. Appendix of male aged 20 years, removed after three slight crises (Obs. 88c). Silvering in the block. Celloidin section.

Argentaffin cells singly and grouped in the subglandular tissue. All the cells are enclosed by nerve filaments (see next Figure). Several chromo-argentaffin cells in the gland epithelium. $\times 46$.



1



2

PLATE 45

FIG. 3. Budding; initial stage (Obs. 88c). Silvering of the section; ponceau-acid fuchsin-aniline blue.

Above and to the left, the bottom of a gland of Lieberkühn. Below and in contact with it nerve filaments of the periglandular plexus. The filaments cut in various planes are surrounded by a collagen sheath, black in the photograph but blue in the preparation. Within this sheath is seen the neuroglia, gray in the Figure, red in the preparation. In cross-sections of the nerve fibers the neuroglia presents a uniformly alveolar appearance. In longitudinal section the alveoli are long, intercommunicating and tubular, running in the direction of the length of the nerve. Inside of these tubes is the pale pink, homogeneous protoplasm of the neurites. At this point there is no hypertrophy of the plexus. Note how much richer it is than in the rest of the intestine (*cf.* Fig. 1). Between the nerves are lacunae of lymphoid tissue. The gland of Lieberkühn is sprouting a bud, not very prominent as yet, formed by a syncytium with four nuclei. The cytoplasm is homogeneous and without granules. $\times 1100$.

FIG. 4. Budding; initial stage (Obs. 88c). Silvering of the sections; ponceau-acid fuchsin-aniline blue.

Here the bud projects directly into a large nerve; the collagen sheath of the nerve is directly continuous with the basement membrane of the gland. There are still no granules in the cells. $\times 1100$.

FIG. 5. Budding more advanced (Obs. 88c). Silvering of the sections; magenta-picricarmin.

Three nuclei of the bud are surrounded by an undivided cytoplasm in continuity with the lining of the gland. Only the cell which has advanced farthest into the nerve is individualized and granular. Nerve plexus hyperplastic; filaments numerous and broad. Above and to the left, an isolated nerve containing a single argentaffin cell. $\times 1100$.

FIG. 6. Budding more advanced (Obs. 88c).

Multicellular bud in a much enlarged nerve. The four cells which migrated earliest into the nerve are individualized and granular; the others, still attached to the gland epithelium, form a multinucleated syncytial mass. $\times 1100$.



3



4



5



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PLATE 46

FIG. 7. Budding more advanced (Obs. 88c). Trichrome.

Swollen bud in a very hyperplastic nerve plexus. The bud is attached to the gland epithelium by a narrow pedicle. Only the two cells farthest advanced into the nerve are individualized and granular. $\times 1100$.

FIG. 8. (Obs. 88c). Trichrome.

On the right, above and below, tips of glands of Lieberkühn. The left of the Figure represents the subglandular region of the reticulated tissue of the mucosa. At one side of the upper gland is a swollen nerve containing two argentaffin cells. From this same gland hangs a bud with slender pedicle ready to part. The bud is enclosed completely in a nerve bounded by its sheath. At several points the cells of the bud are separated from this sheath by neuroglia of spongy appearance. All cells are granular except those of the pedicle. Lower, argentaffin cells detached from their gland matrix and grouped around a cavity full of colloid (vesicle). These cells are enclosed in a hypertrophied nerve, their cytoplasm is continuous without clear demarcation from the neuroglial cytoplasm. $\times 1100$.

FIG. 9. End of budding; differentiation of the argentaffin cells (Obs. 88c). Silvering of the section; ponceau-acid fuchsin-aniline blue.

Swollen bud in the gland plexus. The pedicle is formed by a large hydropic cell with an elongated nucleus staining homogeneously. Seven argentaffin cells are grouped around a cavity full of colloid. Around them and especially below them are seen elongated cells, their protoplasm containing a few argentaffin granules; they are separated from the rosette and seem to be incorporated in the neuroglia. $\times 1100$.

FIG. 10. Vesicular group detached from the gland epithelium. Differentiation of the cells (Obs. 88c). Trichrome. Colored photograph.

In the center, a vesicle with a small cavity filled with colloid and bordered by tall cells, two of which reproduce exactly the form of the chromo-argentaffin cells of the intestinal epithelium. On the right a triangular chromaffin cell having no connection with the cavity of the vesicle. Above the spherical nucleus an angular figure (Nissl body?). Below and on the left cells difficult to identify of ganglionic or neurocrine appearance. Below transition forms between argentaffin and neuroglial cells, elongation of the nuclei, effacing of cellular contours, disappearance of the granules, appearance of intracytoplasmic tubular cavities. $\times 1800$.



7



8



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10

PLATE 47

FIG. 11. Neurocrinia (Obs. 167e). Formol-picric fixation; Marchi's fluid, 8 days. Magenta-picro-indigo-carmin.

Group of neurocrine cells in a hypertrophied nerve. Cells finely granular, contours indistinct, the cytoplasm continuous with that of the neuroglia. In the cytoplasm appear droplets of fat (blackened with osmic acid) which grow larger and escape from the argentaffin cells, being eliminated into the nerve where they soon disappear. They are found only in the immediate vicinity of the argentaffin cells. $\times 1800$.

FIG. 12. Vesicle of argentaffin cells isolated in a greatly hypertrophied nerve filament (Obs. 568 ND). $\times 1100$.

FIG. 13. Two argentaffin cells (neurocrine or ganglionic) enclosed in a nerve (Obs. 81c). Impregnation with reduced silver.

On both sides of the cells are seen four neurites which have taken the silver; it is impossible to see their relations with the cells on account of the black granules which fill the cells.

FIG. 14. Ganglion of the intramucous plexus invaded by argentaffin cells (Obs. 88c). Trichrome.

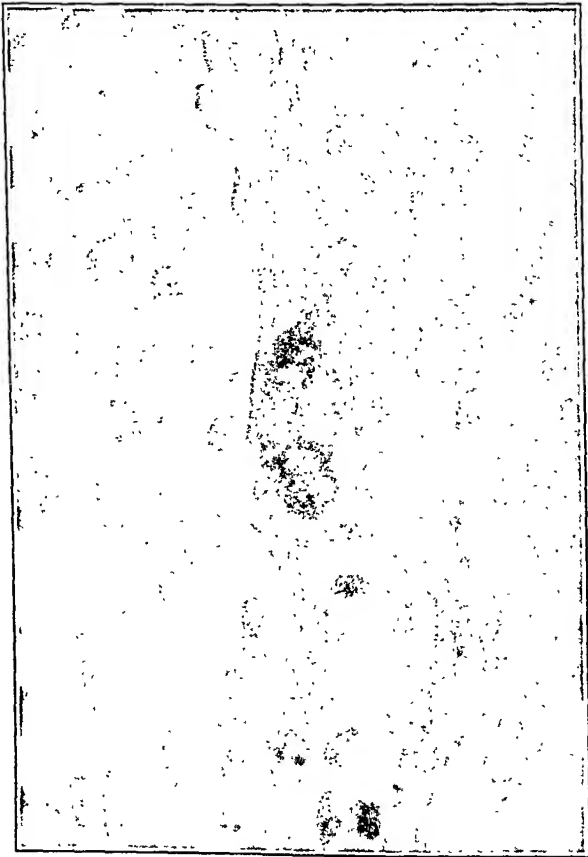
Below and on the right, two non-granular ganglion cells; on the left, two neuroglial nuclei. Above, vesicle of argentaffin cells. In the nerves which leave the upper border of the ganglion, scattered argentaffin cells. In the left upper corner, the edge of a gland of Lieberkühn from which these argentaffin cells have escaped. $\times 1100$.



11



12



13



14

PLATE 48

FIG. 15. Connections of certain intestinal cells with the nerves persisting after budding (Obs. 88c). Silvering of the sections. Magenta-picro-indigo-carmin.

On the right and above, tip of a gland (lumen of the appendix on the right). From this tip (on the left of the Figure) hangs an intranervous bud in full activity. Below and on the right, three cells of the gland epithelium are continuous with the neuroglia of the plexus by means of slender prolongations. Near the prolongations, a round argentaffin cell. It is probable that this cell was recently detached from the epithelium at the very point where the cylindrical cells are in continuity with the neuroglia.

Such views show the actual and direct continuity of certain epithelial cells with the plexus. They show besides that the buds grow from the gland toward the nerve and that the process cannot be an immigration of hitherto intranervous cells into the gland (Danisch). $\times 1100$.

FIG. 16. Neuroma formed in the depth of the mucosa, pushing before it the muscularis mucosae and the submucosa (Obs. 81c). Trichrome. Light area, the neuroma. $\times 48$.

FIG. 17. The deep region of this neuroma.

On the left, in black, the muscularis mucosae. On the right, the neuroma. Above, an argentaffin cell of ganglion type in a node of the neuromatous plexus. $\times 400$.

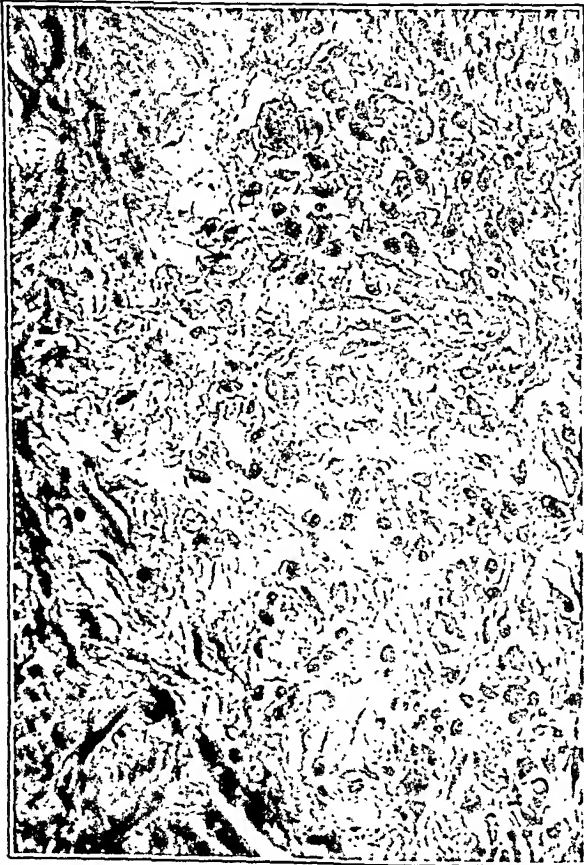
FIG. 18. Another neuroma from the same specimen, 81c. Impregnation with reduced silver. $\times 400$.



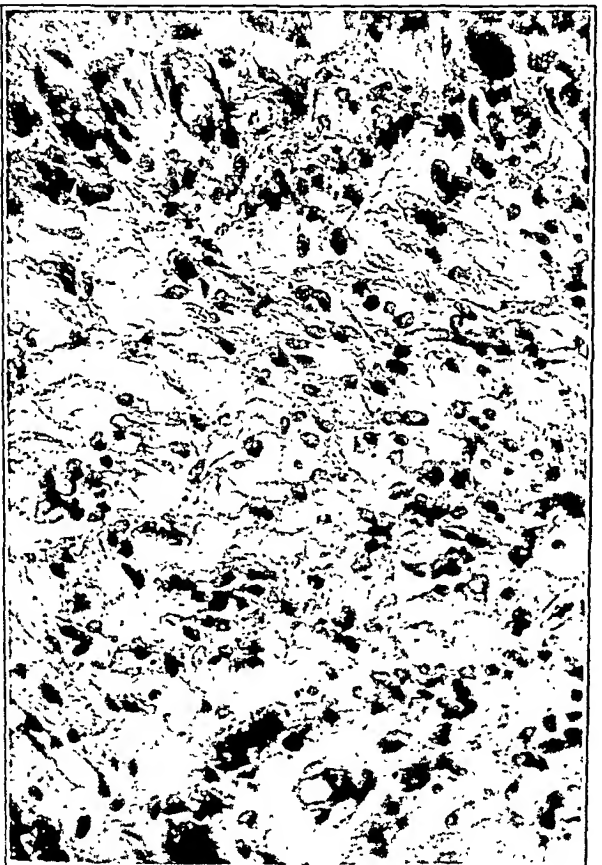
15



16



17



18

PLATE 49

FIG. 19. Appendix partially stenosed. Lateroterminal neuroma in process of growth (Obs. 228f). Trichrome.

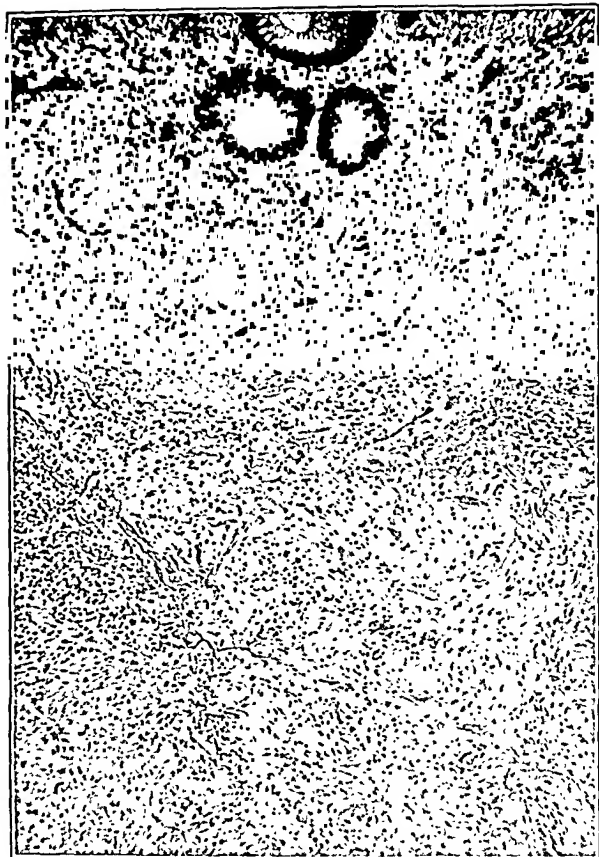
Throughout its permeable portion, the appendix contains tiny intramucous neuromata similar to those of Figs. 16, 17 and 18. At the end of the permeable region, one of these neuromata has grown quite large (light triangle occupying two-thirds of the Figure). This neuroma contains many argentaffin cells. $\times 48$.

FIG. 20. An area in the same neuroma. $\times 800$.

FIG. 21. Appendix completely stenosed (see Fig. 23). (Obs. 773c.) Large axial neuroma. Below and on the right, lymphoid mass representing a neuroma in regression (see Fig. 26). Trichrome. $\times 48$.

FIG. 22. Central region of the same neuroma.

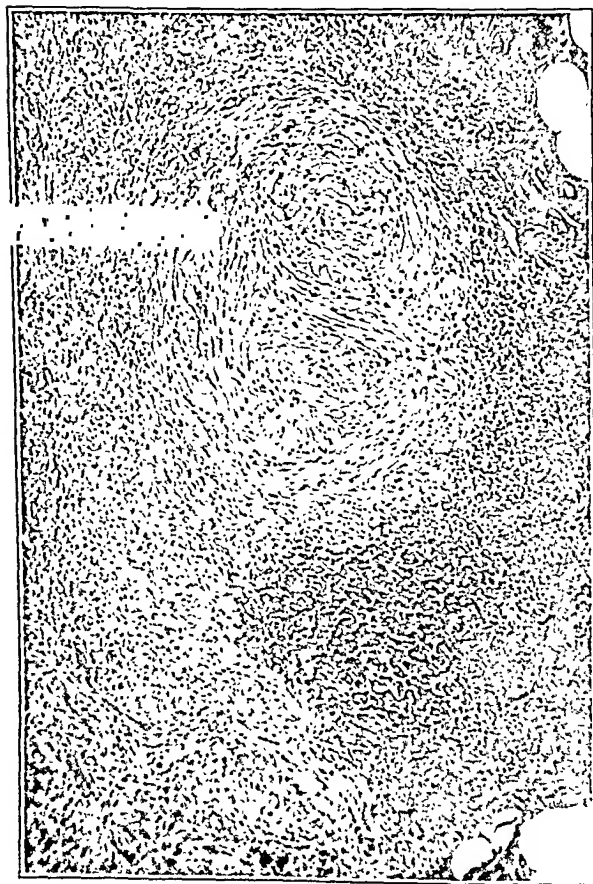
Non-medullated fibers cut across and lengthwise, anastomosing in a compact plexus. $\times 800$.



19



20



21



22

Masson

Carcinoids (Argentaffin-Cell Tumors)

PLATE 50

FIG. 23. Appendix completely stenosed, distal end. Chain of neuromata formed in the axis (Obs. 773c). Trichrome.

Nerve nodules linked transversely and longitudinally by innumerable nerve filaments. In the center of the upper left neuroma, an argentaflin vesicle. The two lymphoid masses represent neuromata without argentaflin cells on the road to resorption. $\times 100$.



PLATE 51

FIG. 24. Argentaffin vesicle (neuro-epithelial rosette?) in the neuroma of Figs. 21 and 22 (Obs. 773c). Trichrome.

The rosette is filled with colloid. Its cells are buried in the neuroglia. The black fibers represent the incomplete collagen sheaths of the nerve fibers anastomosed in an inextricable plexus. $\times 800$.

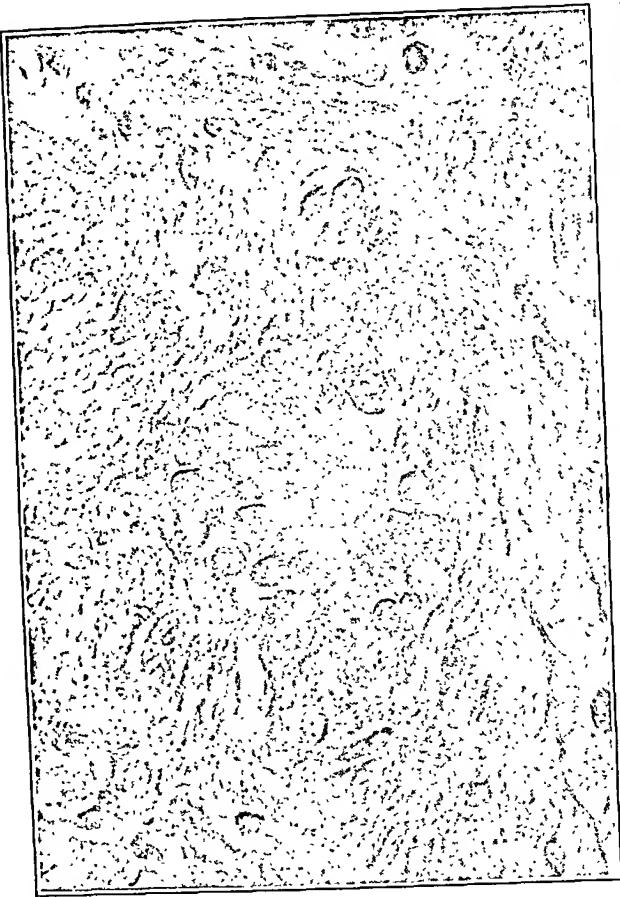
FIG. 25. Longitudinal nerve fibers linking neuroma nodules situated above and below but outside of the Figure (Obs. 69d). Impregnation with reduced silver. Here the silver has stained the neuroglia electively but not the neurites. $\times 400$.

FIG. 26. Degenerating neuroma (Obs. 773 c). Trichrome.

Some fibers recognizable, more or less altered and separated from one another by diffuse lymphoid infiltration. $\times 800$.

FIG. 27. Neurocarcinoid. Trichrome.

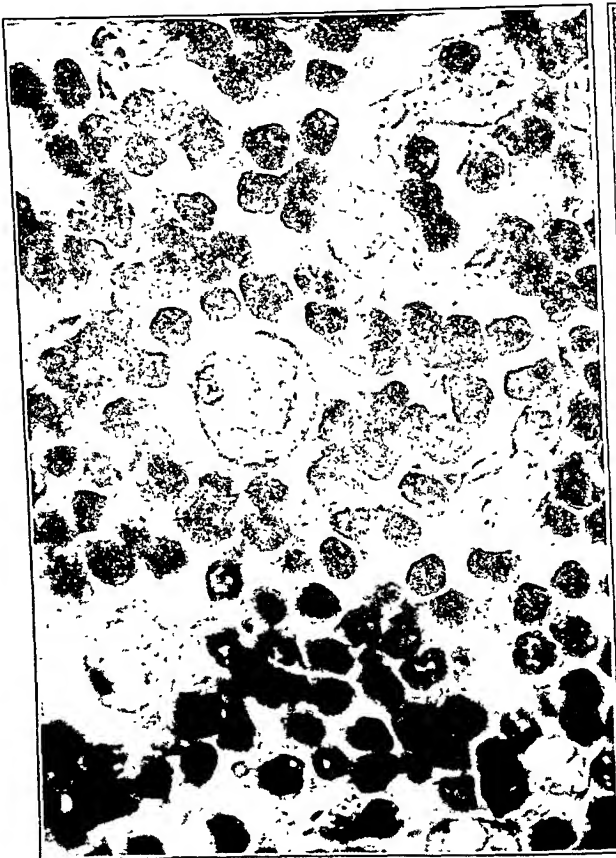
Terminal carcinoid from a child 9 years of age. Spherical tumor, 3 mm. in diameter, formed at the stenosed end of the appendix. The margin of the tumor is invading the submucosa; its center is formed by an axial neuroma. Above are seen the plexiform fibers of this neuroma. Below, these fibers are distended with neurocrine cells which are continuous with carcinoid columns, invading the connective tissue of the submucosa. $\times 800$.



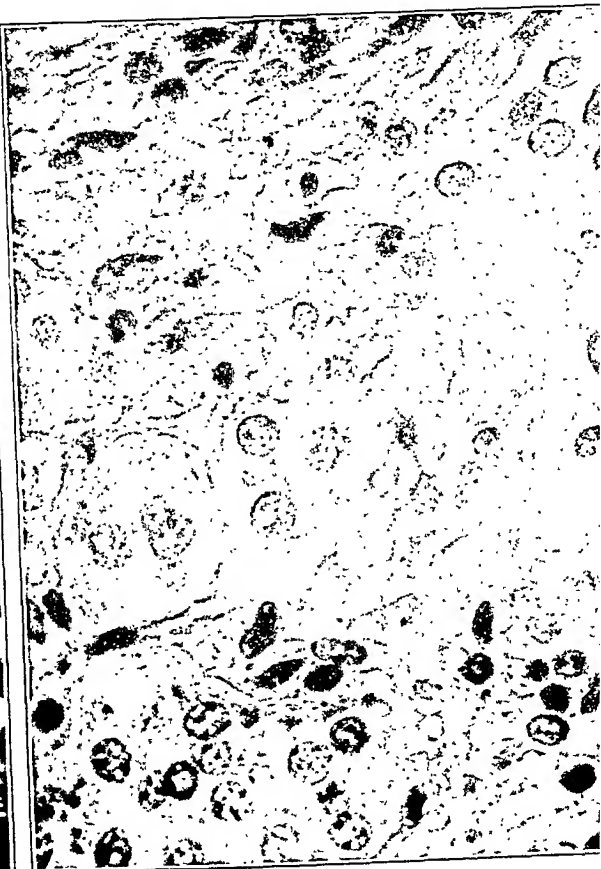
24



25



26

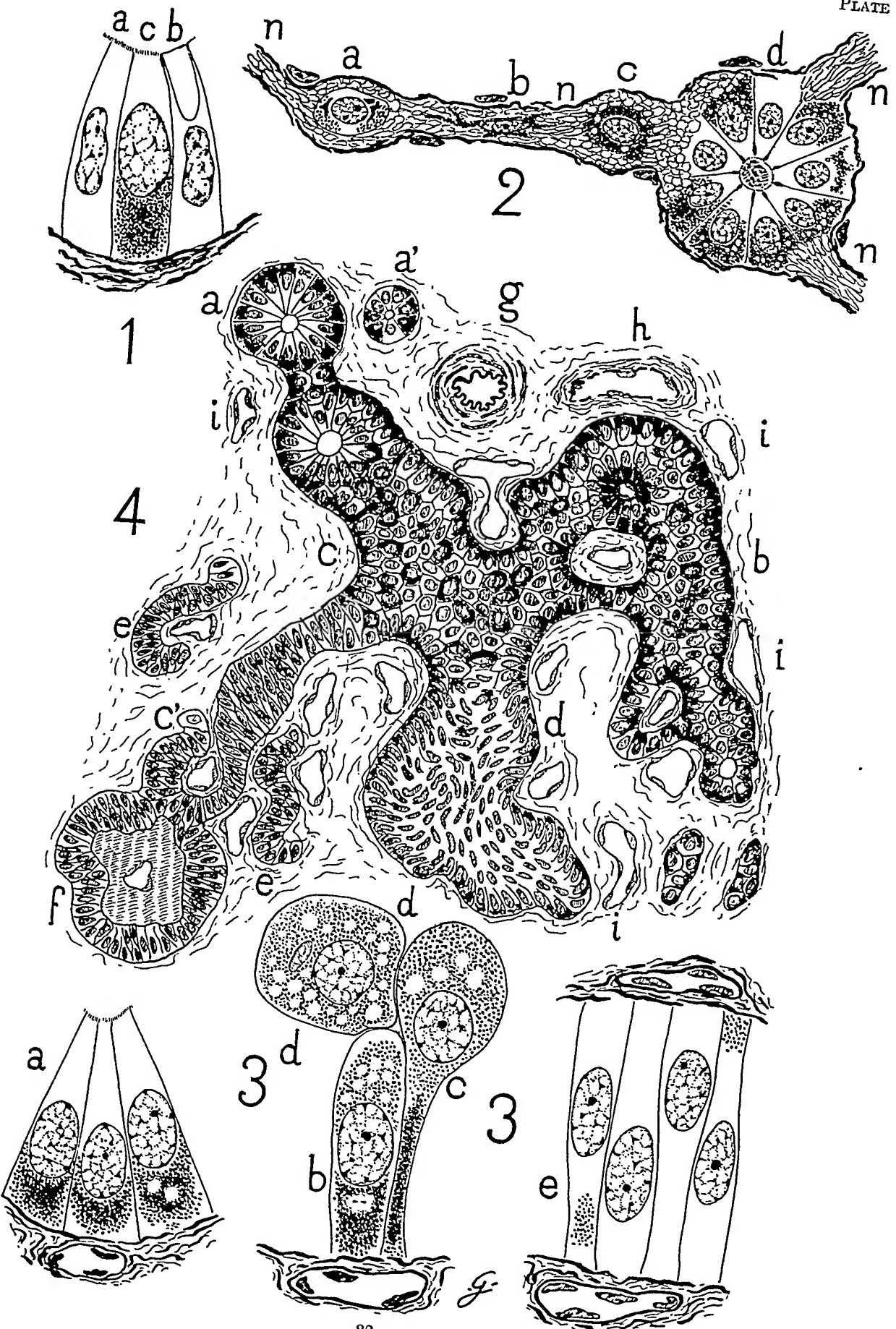


27

PLATE 53
Recapitulation

FIG. 32. 1. Tip of a gland of Lieberkühn: (a) "indifferent" cell; (b) caliciform cell; (c) Kulchitzky cell.

2. Neuroma cells: (*n*) nerve; (a) cell of ganglion type; (b) cell of neuroglia type; (c) neurocrine cell; (d) cylindrical cells of intestinal type (Kulchitzky) forming a vesicle.
3. Carcinoid cells: (a) cylindrical cells of intestinal type (Kulchitzky) forming vesicles; (b) cylindrical palisade cell, the basal region very granular, containing a diplosome, the upper end vacuolated (lipoids) but little granular; (c) racket-shaped cell, swelling of the upper end, pointed at the basal end; (d) polygonal cell, showing central nucleus, parasome, argentaffin granules distributed diffusely and lipid vacuoles. When numerous, these vacuoles give to the cell a spongy appearance; (e) cells from a pure palisade column, granules few, unevenly distributed, at only one pole of the cell.
4. Continuity of different forms of cell columns in carcinoids: (a) column with vesicles of cylindrical cells; (a') column with vesicles of small cuboid cells; (b) column of polygonal cells; (c) palisade and polygonal cell column; (c') penetration of vessels into the palisade and polygonal cell column, angiotropism; (d) column of palisade and fusiform cells; (e) pure palisade column (note relation with capillaries); (f) mucoid imbibition of the connective tissue, cylindromatous evolution; (g) arteriole; (h) venule; (i) capillaries.



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Carcinoids (Argentaffin-Cell Tumors)

THE PATHOLOGIC ANATOMY OF TULAREMIA IN MAN *

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In the publication by Francis and Callender,¹ April 1927, of a study of the microscopic changes in tularemic lesions of man three autopsies are recorded. Other than these we have found no record of a post-mortem investigation of the human disease. In two of the recorded autopsies secondary acute infections were present. This, however, did not mask the lesions of tularemia.

The first autopsy was that reported by Dr. J. R. Verbrycke² in 1924. The post-mortem examination was made by Dr. Maurice Sellinger upon the body of a patient who died eighteen days after the onset of illness. The lesions were therefore early. The second case, that recorded by Dr. Francis,³ died twenty-six days after the onset of illness. The third case, examined by Dr. A. J. Bruecken and reported by Francis and Callender, died about five months after a presumptive exposure to the disease. The case reported by Dr. Francis contracted the disease from the bite of a fly; the other two were apparently caused by handling wild rabbits.

In the first case macroscopic lesions attributable to tularemia were found in the lungs, peribronchial lymph nodes and spleen; in the second, lesions were noted only in the spleen; in the third case infected lymph nodes had recently been removed surgically from the left axilla, and at autopsy tularemic lesions were found in the lungs, peribronchial lymph nodes, liver, spleen, and a lymph node beside the cystic duct. No initial cutaneous lesion was described in any of the three cases.

In addition to these autopsies tularemic lesions have been studied in the conjunctiva,⁴ in subcutaneous tissue,⁵ and in lymph nodes in material removed surgically. Francis and Callender described the lesions in surgical specimens from seven cases.

An initial ulcer of the skin, which is frequently associated with the disease, has not heretofore been described histologically. It was the primary lesion in the case to be reported.

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From published studies it is found that the early lesions in the lymph nodes, lungs, spleen and liver are focal areas of necrosis with a cellular reaction consisting of epithelioid cells and polymorphonuclear leucocytes. When these areas undergo caseation, they resemble tubercles very closely. Langerhans' cells have frequently been observed about the margins of the necrotic areas. The lesions tend to be chronic, and healing is indicated by the ingrowth of fibroblasts about the margins, finally encircling the necrotic focus with a fibrous capsule. Suppuration of superficial nodes is described.

Individually the lesions are often not to be distinguished from tubercles. Evidence of their non-tuberculous nature is the absence of tubercle bacilli in stained preparations, and the failure of guinea pigs, inoculated with material which has been subjected to conditions which destroy *B. tularensis* but not the tubercle bacillus, to contract tuberculosis.¹ The true nature of the infection in man has in some cases been demonstrated by inoculating rabbits, guinea pigs or white mice, which are very susceptible to tularemia, with infectious material, and by the appearance in the patient's blood of specific agglutinins ten days to two weeks after the onset. The *Bacterium tularensis* has never been demonstrated in smears or sections of human tissue. Nor is it always demonstrable in similar lesions of lower animals. The microorganism, however, may be readily cultivated from suitable material by the use of proper media.

There are many ways by which the disease may be transmitted from an infected host to man. By far the greatest number of such transmissions follow the handling of infected rabbits, and begin with an indolent ulcer which develops at the site of an abrasion on the hand. Primary opthalmic tularemia may also follow similar exposures. Other modes of transmission are by the bite of the wood tick and the deer fly (*Chrysops discalis*), an ulcer developing at the site of the bite. The disease has been known to occur, following the handling of infected rabbits, without the development of a local lesion. Ohara⁶ proved by human inoculation that such could occur. There is a typhoid form of the disease, the type usually presented by laboratory workers who contract it from handling infected animals. In this form there is usually no local lesion. Droplet infection⁷ may play a rôle in some of these cases.

Whether or not specific agglutinins invariably appear has not

been proved, but it is assured that they may be absent in early stages of very acute fulminating forms of the disease.

In human infections a history of a wound, received while handling wild rabbits, followed by an indolent ulcer on the hand at the site of the injury, inflammation of lymph nodes draining this area, and a febrile reaction, offer good presumptive evidence of a tularemic infection. A positive agglutination of *B. tularensis* by the patient's serum serves to confirm the diagnosis.

The mortality from the disease is relatively low, namely, about three per cent.

During the past fall and early winter thirty-one cases of tularemia have been reported in Davidson County, Tennessee. A considerable number of cases have been observed also in neighboring counties, and all of these, so far as we have been able to determine, have been due to contact with wild rabbits. Only one fatality has come to our attention. This was a typical case of the ulcero-glandular type which ran a violent course terminating in death after fifteen days. An autopsy was performed within an hour after death, and the study of this material is the basis of our report.

REPORT OF CASE

Clinical Report: A. T. R., a white, male, American, aged 29 years, presented himself for examination on December 1, 1927, complaining of a painful swelling in the right axilla, general malaise, and a sore finger on the right hand.

Past Illnesses: On September 26, 1927, the patient became ill with typhoid fever. He had fever for about three weeks and made a fairly satisfactory convalescence. He left the hospital, under protest, on October 20, 1927, about one week after being afebrile.

Occupation: About November 15, 1927, he secured a position with a produce house, dressing fowl and rabbits.

Present History: On November 26, 1927 while dressing rabbits the right index finger was scratched. On November 28, while dressing rabbits the right index finger was injured again with a rabbit bone. On November 29, there was a small, painful swelling of the injured finger. On November 30 soreness was noted in the right axilla. On December 1, as stated, the patient presented himself for examination.

Physical Examination: There is slight evident pallor; temperature 101° F; pulse 100; respiration 20. There is an area of redness and induration on the dorsum of the terminal phalanx of the right index finger, more marked around the nail. There is a small (2 mm.) crust-covered abrasion in the center of the indurated area. There are no evident red streaks and no glands or tender areas palpable along the arm. There is a very tender mass in the right axilla which on careful examination appears to be an agglutinated mass of enlarged glands. The

entire mass is estimated to be about 8 cm. in diameter. There is a palpable gland in the right supraclavicular fossa about 2 cm. in diameter, and not tender; no other superficial glands are enlarged or tender. The examination is otherwise essentially negative. The leucocyte count is 13,000. On account of the history and findings, a working diagnosis of tularemia was made.

Course: Treatment was carried out at home for two days. Hot wet packs were applied to the finger continuously, and dry heat to the axilla. Aspirin was given q. 4 h. In spite of rather energetic treatment along these lines the patient's fever rose to 103° F, the glands in the axilla continued to swell and the general condition of the patient became progressively worse. He was sent to the hospital on December 3, 1927. At this time about a drop of pus had accumulated at the site of the abrasion in the center of the area of induration; and this was evacuated. On admission to the hospital the leucocyte count was 15,000; the red cell count 3,750,000; the hemoglobin 75 per cent. The differential count showed 72 per cent polymorphonuclear leucocytes, 16 per cent small lymphocytes, 6 per cent large lymphocytes, 5 per cent mononuclears, and 1 per cent eosinophils. Two days later the leucocyte count was 19,000; the urine was negative. A few hours after admission nausea and vomiting occurred and continued throughout the illness. The patient complained of pain in the head, throat, neck, back, joints, and abdomen. The teeth became very sore, and there was an extremely bad taste in the mouth. On December 4, the temperature and pulse returned to normal for a few hours during which time the patient seemed better. The following day the temperature reached 104° F. On December 9, the mass of glands in the right axilla was only about 3 cm. in diameter, and not very tender. On this day the temperature again came to normal for a few hours without any apparent improvement in the patient's condition, and there was more pain than usual in the abdomen. Examination revealed marked rigidity and tenderness in the right upper quadrant. It was thought that much of the rigidity was due to an enlarged liver, the lower edge of which was not palpable. On the following day, however, it was felt with more certainty that the liver was greatly enlarged. The spleen also became palpable on this day and was very firm. Treatment necessarily remained symptomatic, and this was of little avail. The patient became progressively weaker. On the night of December 11, twelve days after the appearance of the lesion on the finger, blood was drawn and kept in the ice-box over night. The serum from this sample was divided and half of it was sent to Dr. Francis at Washington, who reported that it did not agglutinate *B. tularensis* (this serum was inactivated before being mailed). The other half, without being inactivated, was sent to Dr. R. L. Jones, of the City Health Department, who reported positive agglutination in dilutions of 1:40 and 1:80 with *B. tularensis*.

On December 13, the patient's condition was most critical, it being evident that the end was very near. A transfusion of 300 cc. of citrated blood was introduced into the patient's vein. Two and one-half hours after transfusion he died. The patient lived fifteen days after receiving the second injury on his finger. A few hours before death the leucocyte count had risen to 22,600.

PATHOLOGIC REPORT

The body was that of a young white man, well developed and well nourished. It was still warm and rigor mortis had not set in. The

head, eyes, ears, nose and mouth presented no noteworthy abnormalities. The thyroid gland was not enlarged. There was no edema.

On the dorsal surface of the terminal phalanx of the right index finger next to the nail there was a circular ulcer 8 mm. in diameter. The base was about 1 mm. deep. It had a brownish, opaque, necrotic surface. There was very little evidence of inflammation about the margins. The ulcer was excised for histologic examination.

The axillary lymph nodes on the right were easily palpable and distinctly enlarged. A supraclavicular node on the right was palpable. The nodes in the left axilla were not felt, and there was no enlargement of the inguinal nodes.

Peritoneal Cavity: On primary incision subcutaneous fat was presented in moderate amounts. The musculature of the abdominal wall was well developed and of reddish color. The peritoneal surfaces presented a smooth, shiny, moist appearance. There was only a slight increase of peritoneal fluid. The abdominal organs were normally situated. The liver was very much enlarged and extended two fingers breadth below the costal margin in the right mamillary line. The liver edge was firm and rounded. The spleen also was considerably enlarged. The appendix appeared normal. The omentum was fatty and free of adhesions. The stomach was contracted. Greatly enlarged lymph nodes were observed along its lesser curvature and extending into the hilum of the liver.

Thoracic Cavity: On removal of the sternum the lungs were observed to be moderately voluminous and pink. The anterior mediastinum was fatty and contained several enlarged lymph nodes. Enlarged nodes extended from the mediastinum upward into the deep tissues of the neck on either side.

Pleural Cavities: There were about 200 cc. of clear fluid in the left pleural cavity. On the surface of the right pleura, where it adhered to the superior mediastinum mesially, there was a patch of pinkish gray granulations, evidently a localized fibrinous pleuritis adjacent to the mediastinum. A great mass of lymph nodes could be felt about the bifurcation of the trachea, and in the hilum of each lung.

Pericardial Cavity: There was a normal amount of clear, straw-colored fluid in the pericardial sac. The pericardial surfaces were smooth, shiny and normal in appearance. The heart did not appear to be enlarged, or grossly abnormal.

Heart: Weight 350 gm. The valves, endocardium, and coronary arteries presented no noteworthy abnormality. The myocardium was somewhat pale and more opaque than normal.

Lungs: The lungs were everywhere air-containing, except for a superficial zone in the left lower lobe posteriorly where there was atelectasis. There was no evidence of consolidation or inflammation in either lung. The bronchial mucous membranes were pink and normal in appearance. There was, however, a large mass of firm lymph nodes about the bronchi and in the hilum of each lung, and a chain of similar enlarged edematous nodes extended along the esophagus and below the diaphragm. These will be described more in detail below.

Gastro-Intestinal Tract: The stomach was practically empty. The mucosa of the esophagus, stomach, and the small and large intestine throughout presented a normal appearance. Bile could be expressed through the ampulla of Vater with a little pressure on the gall-bladder. The chain of lymph nodes along the lesser curvature of the stomach extending into the hilum of the liver was greatly enlarged. There was a similar enlargement of the periaortic nodes of the upper abdomen. The mesenteric and lower abdominal nodes were not enlarged.

Liver: The liver weighed 3100 gm. It was very large, smooth and rounded. It had a yellowish, mottled appearance. The individual lobules appeared enlarged, and on section here and there tiny gray dots like miliary tubercles could be seen by careful scrutiny. The consistency was fairly firm. The gall-bladder was distended with bile. The lymph node beside the cystic duct was conspicuously enlarged, and in the hilum there was a mass of very large nodes, to be described in detail below.

Pancreas: The pancreas was of normal size, color and consistency. Above and about it were numerous enlarged lymph nodes.

Spleen: The spleen was greatly enlarged weighing 950 gm. It had a smooth surface, a fairly firm consistency and a dark purple color. Through the stretched capsule could be seen several light gray areas measuring up to 1 cm. in diameter. On section the cut surface presented a unique appearance. Very numerous chalky white, or yellowish opaque spots measuring up to 1 cm. produced a striking effect. The larger were irregular in outline almost angular. Their clean-cut margins and their opaque yellowish or whitish color

contrasted sharply with the dark purple background of the pulp. From the centers of the larger areas a soft pasty material could be scraped out with the knife. The Malpighian bodies were not conspicuous. The areas of necrosis in the spleen do not resemble those of tuberculosis, and the effect they produce is the most characteristic feature of the gross pathology of tularemia. The spleen measured 17.5 by 11.5 by 6 cm.

Kidney: The right kidney weighed 180 gm.; the left 170 gm. The capsule of each stripped easily, leaving a smooth, pale, slightly opaque surface. On section the consistency was normal. The cortex presented normal striations. The cortical zone was thicker than normal, and the slight grayish opacity of cloudy swelling was observed. The pelves and ureters appeared normal.

Adrenals: These glands were of normal size and consistency. On section they presented normal markings.

Pelvic Organs: The urinary bladder and prostate presented no noteworthy alterations.

Testes: The left testis appeared normal. The right testis was small, firm and fibrous, having on section a yellowish, opaque appearance, as if partially necrotic and fibrosed.

Brain: The central nervous system was not examined.

Bone Marrow: The marrow of the ribs and right femur was abundant and pink.

Lymph Nodes: The group of lymph nodes in the right axilla draining directly the ulcer of the right index finger were removed. Several of the nodes were considerably enlarged measuring up to 1.5 cm. It was stated that from clinical examination they had diminished in size following great initial swelling. They were discrete and fairly firm. On section the cut surface presented numerous yellowish, opaque spots about 1 mm. in diameter, uniform in size, and regularly distributed throughout the nodes. Beneath the capsule were streaks of a similar, opaque, yellowish color corresponding to the peripheral lymph sinuses. The content of these areas was pasty, but not fluid or purulent. The necrotic material had the appearance and consistency rather of caseous matter, and altogether the process had a considerable similarity to a tuberculous lesion. Each of the enlarged glands from the axilla presented a similar appearance. An enlarged supraclavicular node showed no areas of necrosis on section.

Lymph nodes from the anterior mediastinum and the deep tissues of the neck on both sides presented an appearance similar to that in the nodes from the right axilla. The nodes at the hilum of the lung were bound together by an intervening rather dense fibrous tissue. On section through these masses, the discrete outlines of anthracotic nodes were readily discernible, and their cut surfaces presented likewise the discrete opaque caseous spots. The mass of hilic glands measured 7 by 6 by 4 cm.

The nodes along the esophagus and aorta were discrete and greatly enlarged. On section they were somewhat softer and more edematous than those above. The cut surfaces were mottled by grayish and yellowish areas of necrosis which were larger, more extensive and less of a caseous appearance and consistency than those in the right axillary nodes. These areas had the appearance of a more recent coagulative necrosis, less well circumscribed and containing and being surrounded by a more fluid exudate. This was true also of the nodes along the lesser curvature of the stomach, pancreas and hepatic hilum. In these nodes, which measured up to 4 cm., the necrosis appeared most recent and extensive. All of these nodes were discrete.

REMARKS

The infection evidently is preëminently a lymph borne one, extending from the index finger to the right axillary nodes, and thence to the mediastinum, peribronchial and hilic regions, thence upward into the neck and downward along the esophageal and aortic nodes to the upper abdomen, and across through the gastric nodes into the hilum of the liver. Hemal distribution is indicated only by the areas of necrosis in the spleen and the miliary foci in the liver. Grossly the lesions individually in the axillary nodes are indistinguishable from tuberculous caseation. Their uniformity in size, age, and distribution in these nodes is not like tuberculosis, nor is the more recent necrosis of the abdominal nodes. The distribution along the lymphatic chains as presented in this case is again unlike tuberculosis. Also the lesions in the spleen with their chalky appearance, their irregular outline and absence of encapsulation present a picture which is to be regarded as certainly unusual for tuberculosis. Thus, while the lesions individually are very similar to tuberculosis, the pathologic process as a whole has in this case at least a certain distinctiveness.

HISTOLOGIC REPORT

Heart: There is a marked congestion and interstitial edema of the myocardium of the left ventricle. Cloudy swelling is present. About some of the veins there is a moderate accumulation of large mononuclear cells.

Lung: A section through a primary bronchus, with a small lymph node attached, shows a small hyalinized healed tubercle in the node.

Aside from areas of atelectasis associated with edema in certain portions, the sections of lung present no noteworthy changes.

The pleura from the upper mesial surface of the right side which showed a granulating appearance in gross, presents a subacute inflammation. The surface is covered with fibrin, within and below which are polymorphonuclear leucocytes and epithelioid cells. Beyond these are numerous lymphocytes.

Thymus: A section of thymus shows thin strands of thymic tissue embedded in adipose tissue. No abnormality is noted.

Gastro-Intestinal Tract: Sections through the stomach show a slight polymorphonuclear cell exudate in the mucosa.

Liver: There are numerous focal scars in the liver. Most of them are about the size of an hepatic lobule and fairly definitely circumscribed. Some of these scars in addition to fibrous strands contain numerous large and small mononuclear cells and an occasional isolated hepatic cell. Others are composed almost completely of hyalinized fibrous tissue. An occasional scarred area is quite extensive involving several adjacent hepatic lobules. There is some, but relatively little, regeneration of bile ducts. These scars have the size and distribution of typhoidal lesions, and are considered to be healed areas of focal necrosis.

In addition to the scars there are acute focal lesions undoubtedly of tularemic origin. These are relatively few in number and are irregularly disposed through the hepatic parenchyma. They are of the size and general appearance of small and large miliary tubercles. The earlier ones consist of a focal collection of polymorphonuclear and large mononuclear cells, with little necrosis in the center of the group. Larger ones show central necrosis and caseation with a marginal zone of epithelioid cells. No Langerhans' cells are present.

The hepatic cells are swollen and show the distinct granulation of cloudy swelling. There is considerable congestion.

Pancreas: The pancreas presents no noteworthy abnormality.

Spleen: The splenic pulp is engorged with blood, and much of the blood appears to be extravasated. Great numbers of polymorphonuclear and large mononuclear cells are present, diffusely scattered throughout the pulp. The Malpighian bodies are rather pale and diffuse. Here and there are focal scars, and there is an increase in connective tissue about some of the larger blood vessels. The focal scarring suggests the results of typhoidal necrosis.

Dispersed irregularly through the pulp are large and small areas of necrosis which are evidently of tularemic origin. These areas have necrotic, sometimes caseous centers, with a marginal zone of epithelioid cells. No Langerhans' cells are present. In the center of very large areas of necrosis there is autolysis and partial liquifaction. In smaller areas in which necrosis has not advanced, there are a great number of polymorphonuclear as well as epithelioid cells; and fibrin strands are to be seen.

Adrenals: The adrenals show no noteworthy pathologic changes.

Kidney: The kidneys are congested, and there is conspicuous parenchymatous degeneration.

Urinary Bladder: Sections show no noteworthy pathologic changes.

Testes: One section shows complete atrophy of the seminiferous tubules. There is also hyalinization and atheromatous degeneration of arterial walls. Leidig's cells are not prominent. Another section shows a large infarct, with hyalinization of the infarcted area. Hematoidin crystals are abundant.

Prostate: Sections show no noteworthy pathologic changes.

Lymph Nodes: Sections through the right axillary lymph nodes, which drained the ulcer on the finger, show a subsidence of the acute inflammation. The lymph sinuses are not markedly distended and contain relatively few large mononuclear leucocytes. Corresponding to the opaque yellow spots noted in gross, there are circumscribed areas of necrosis with central caseation and a well developed marginal zone of epithelioid cells. No Langerhans' cells are present. Except for the absence of giant cells these lesions closely resemble tubercles. Here and there a blood vessel in the neighborhood of a caseous focus shows the deposition of thrombus on its intimal surface next to the necrotic area. The thrombus is composed for the most part of fibrin and large mononuclear cells.

Sections through lymph nodes from about the bronchi, esophagus, aorta, stomach, pancreas and hilum of the liver all contain numerous similar lesions but evidently more recent, in that none shows quite so advanced caseation. Various stages in the formation of these lesions may be seen. The lymph node as a whole is edematous and inflamed. The blood vessels throughout are conspicuously dilated and engorged with red blood cells. The lymph sinuses likewise are dilated, containing the granular precipitate of coagulated lymph, numerous large phagocytic cells, polymorphonuclear leucocytes and occasional fibrin strands. Many focal lesions can be seen to originate within a lymphatic space. A network of fibrin is precipitated and in its meshes are the irregularly shaped nuclei of epithelioid cells often associated with numerous polymorphonuclear leucocytes. Other lesions seem to originate in the parenchyma. Here again there is a deposit of fibrin and often large numbers of polymorphonuclear leucocytes are present. The cellular exudate of the foci usually consists of both polymorphonuclear and epithelioid cells, sometimes one and sometimes the other predominating. As the lesion advances necrosis occurs in the center and a marginal zone of epithelioid cells becomes apparent. No Langerhans' cells have been seen in any section, and no lesions older than those in the right axilla have been observed. All of the lesions therefore are acute and necrotizing.

No microorganisms have been demonstrated.

Ulcer of the right index finger (initial lesion): Sections through the center of the ulcer show the base to consist of necrotic tissue which extends deeply. The type of necrosis resembles that seen in the centers of the lymphatic foci, that is, it is a coagulation necrosis resembling caseation. It consists of a pinkish amorphous material containing dust-like particles of fragmented nuclei. In some places nuclei are undergoing karyolysis. About the margins of the necrotic mass there is a zone of polymorphonuclear and epithelioid cells, the one or the other type predominating in different areas. The necrosis extends into the surrounding tissues particularly about arteries, which it tends to surround, leaving the vessel relatively unimpaired in the center. Further out there is a large mononuclear cell and lymphocytic exudate about blood vessels and sweat glands. Sometimes the large phagocytes contain numerous lymphocytes. There is little or no evidence of an acute inflammation in the tissue surrounding the ulcer.

Note: Tissues were fixed in Zenker's solution, Schaudinn's fluid, Helly's fluid and formaldehyde. Paraffin sections were stained, in addition to the routine methods, by Giemsa's, Gram's, the carbolfuchsin, and carbol-anilin-fuchsin methods for bacteria. No microorganisms of any kind were revealed. Smears from the fresh lesions similarly stained showed no microorganisms.

DISCUSSION

The diagnosis of this case rests finally upon the history, clinical course, the pathologic anatomy, and a positive agglutinin test. Rapidly following a scratch on the right index finger, received while dressing wild rabbits, a local ulcer developed. The ulcer expanded slowly and its base became lined by necrotic tissue. There was a relatively slight inflammation about it. The subsequent lymph node involvement is directly attributable to the lesion on the finger. First in order the right axillary nodes became enlarged and painful. There was a progressive infection of lymph nodes extending from the right axilla to the mediastinum, downward along the esophagus through the diaphragm and across the lesser curvature of the stomach into the nodes of the hilum of the liver. Microscopically, the ulcer of the finger presents the same type of necrotizing injury and cellular reaction that is found in the lymph nodes, that is, a cellular reaction consisting of polymorphonuclear leucocytes and epithelioid cells aggregated in masses often in a meshwork of fibrin. The focus undergoes a caseating necrosis. The necrosis is surrounded by a marginal zone of epithelioid cells. Many of the foci in lymph nodes begin within lymphatic spaces, others seem to arise within the parenchyma. The entire node becomes acutely inflamed. Blood vessels are dilated. The tissue is edematous and the lymph sinuses are distended with fluid containing numerous cells, large phagocytes, lymphocytes and polymorphonuclear leucocytes intermingling. The larger phagocytes often contain other cells within their cytoplasm. Thus the infection may obviously spread along lymphatic channels. Through these channels the virus may enter the blood stream. Or the blood stream may be entered directly. Frequently one sees in sections of the lymph nodes an area of necrosis encroaching upon a blood vessel which becomes thrombosed. Sometimes necrotic blood vessels are seen in the center of a focus of necrosis.

The bacteria circulate in the blood at intervals if not continuously and this is made manifest by the occurrence of typical foci of necrosis in the spleen and liver. Grossly the appearance of the spleen seems quite characteristic. It is enlarged, distended with blood, and is speckled on the cut surface with opaque yellowish or chalky areas measuring up to about 1 cm. in diameter, sharply circumscribed, irregular in outline and even with the surface. The larger areas have softened centers.

Clinically the right axillary nodes diminished appreciably in size during the course of the disease, and histologically the general exudative reaction is less marked and the foci of necrosis appear older, that is more compact and caseous, than in the lesions elsewhere.

The serum removed on the 13th day of the disease was reported as agglutinating *Bacterium tularensis* in one laboratory and was negative in another.

Our case showed no evidences of active infection other than those of tularemia. The pathologic anatomy is regarded as characteristic. The infection as illustrated by this example is essentially lymphatic in distribution resembling in this respect bubonic plague, which the disease simulates in rodents.⁸

It is noteworthy that this case died of what appeared to be an intoxication from a pure tularemic infection. No tularemic or other lesions were found in the lungs. Histologically the lesions differ from most of those previously described only in their recentness and in the absence of Langerhans' cells.

SUMMARY

1. The pathologic anatomy of a typical case of ulcero-glandular tularemia studied by autopsy is presented.
2. The histology of the initial ulcer is described for the first time in the human disease.
3. The lesions are essentially lymphatic in distribution affecting many contiguous groups of lymph nodes, the spleen and liver.
4. The individual lesions have a certain similarity to those of tuberculosis, but collectively they constitute a characteristic picture.

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DESCRIPTION OF PLATES

PLATE 54

- FIG. 1. Right axillary lymph nodes. The cut surface shows the discrete gray spots of necrosis. Actual length of specimen 5 cm.
- FIG. 2. Section through a mass of peribronchial lymph nodes. Actual length 6 cm.
- FIG. 3. Peribronchial, esophageal, and subdiaphragmatic nodes, greatly swollen. Actual length 21 cm.
- FIG. 4. Group of enlarged partially necrotic nodes in the hilum of the liver. Note the great enlargement of the node beside the cystic duct. Actual length 16 cm.



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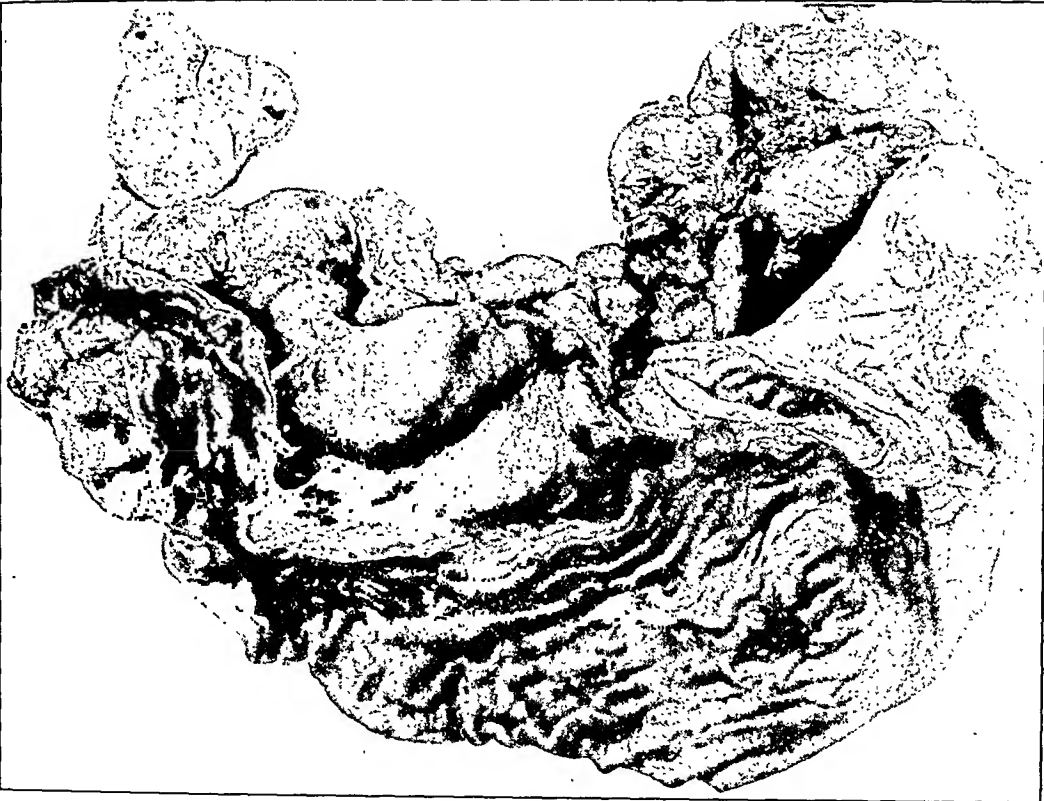


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PLATE 55

FIG. 5. Stomach with pancreas attached. Across the lesser curvature is a great chain of swollen lymph nodes. At the upper left is a cut section through a large one showing pale areas of necrosis. Actual length 16 cm.

FIG. 6. Enlarged nodes in the hilum of the lung. Actual length 16 cm.



5



6

PLATE 56

FIG. 7. Cut surface of the spleen showing the chalky opaque areas of necrosis.
Actual length 16 cm.



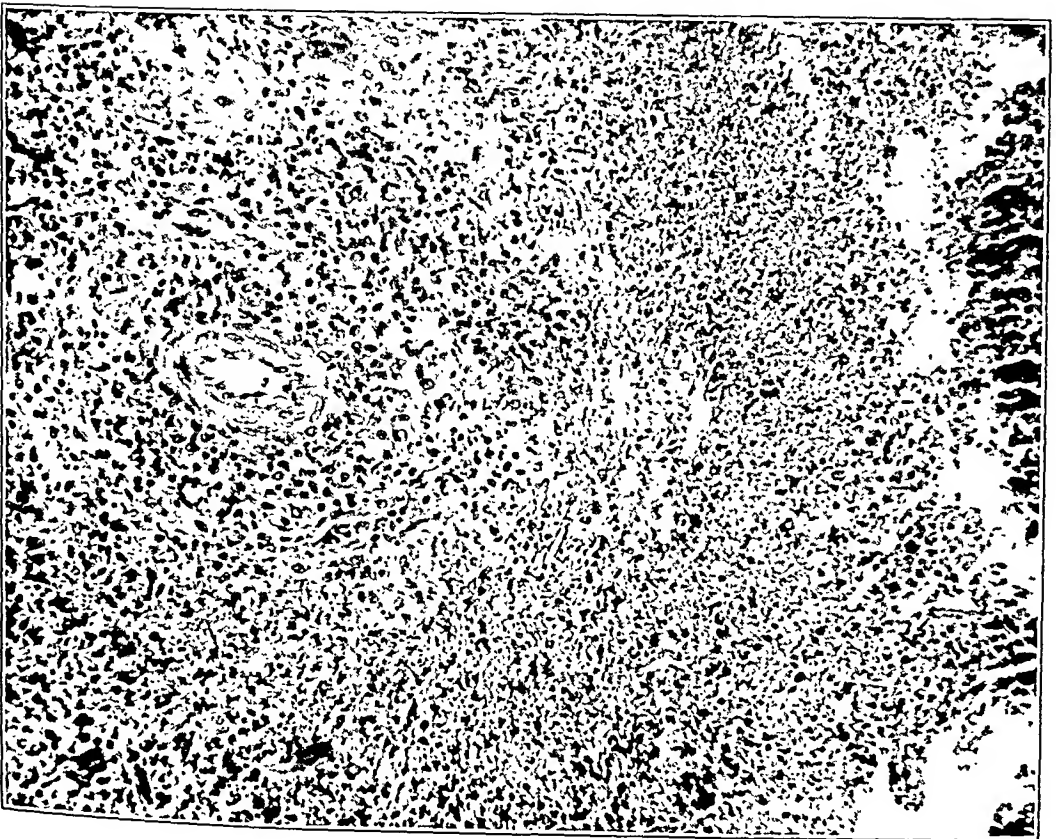
PLATE 57

FIG. 8. Section through the base of the ulcer on the right index finger. It shows the depth of the necrosis and in places its perivascular arrangement. $\times 20$.

FIG. 9. A magnification of the blood vessel at the lower right of Fig. 8, showing caseating necrosis and cellular reaction about a small artery. $\times 200$.



8

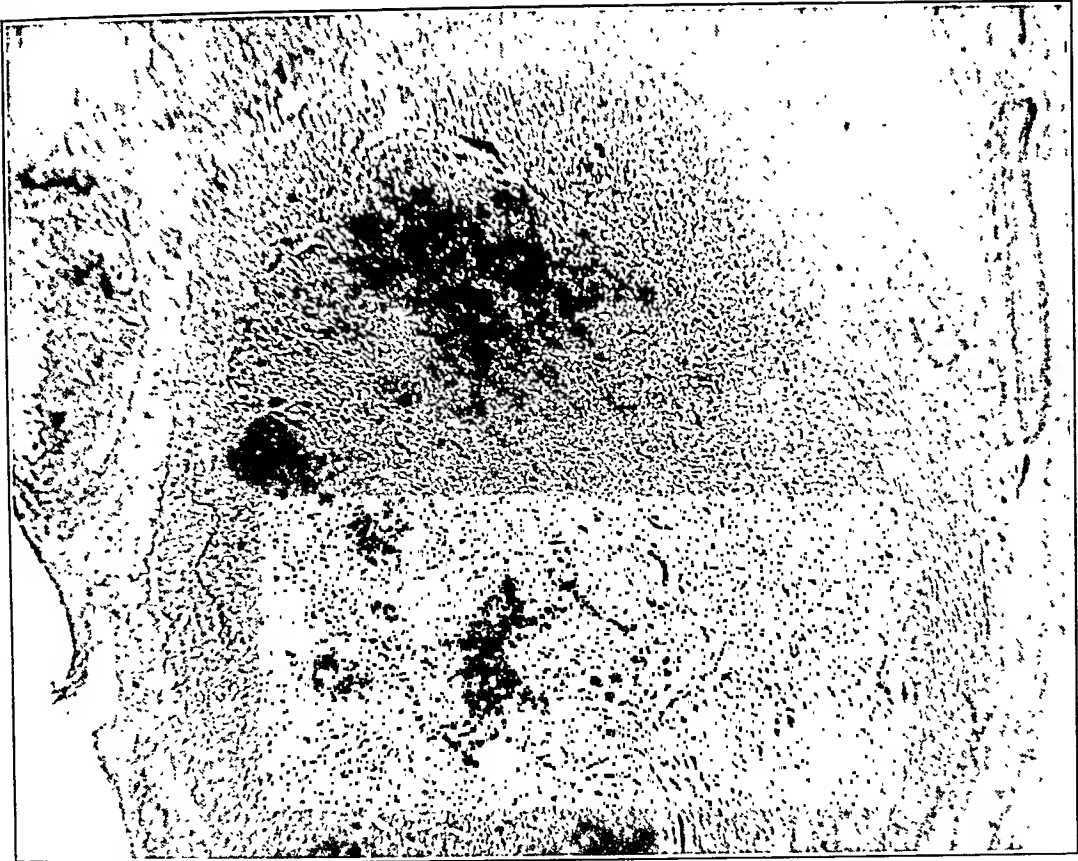


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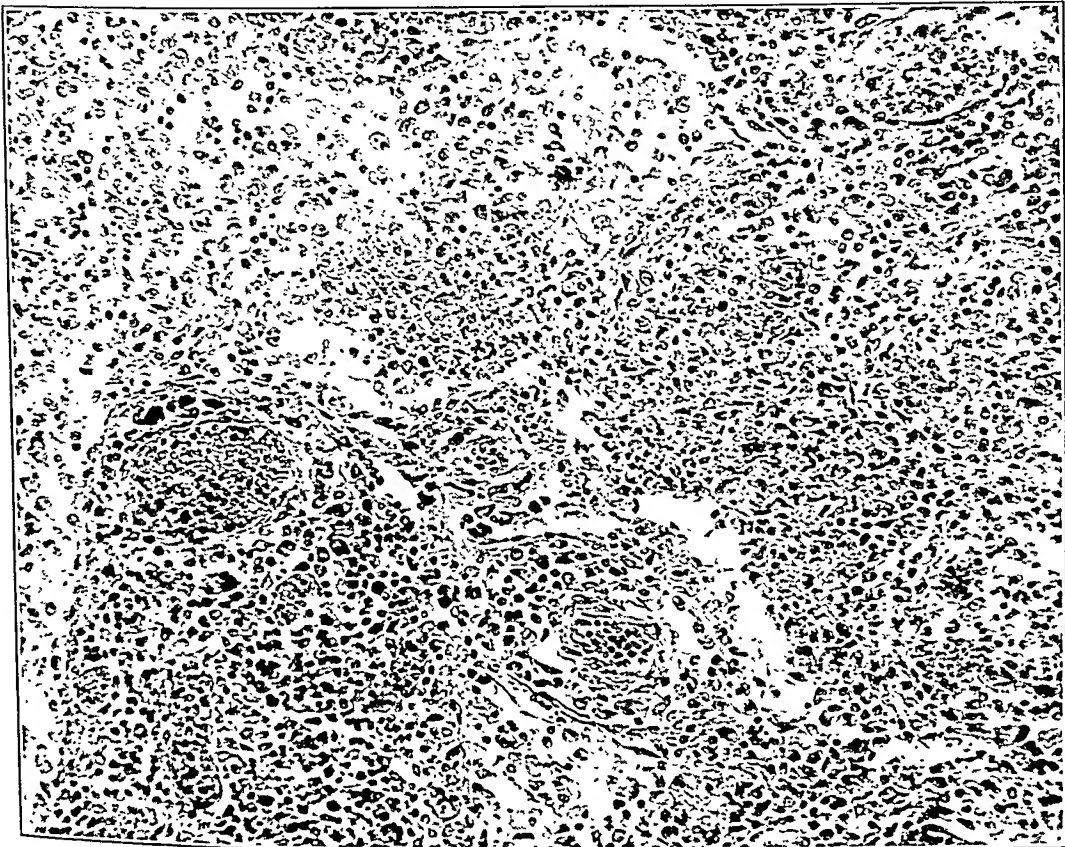
PLATE 58

FIG. 10. Section through a small lymph node showing the extent and distribution of necrosis. $\times 20$.

FIG. 11. A lymphatic sinus filled with cellular exudate. A cellular thrombus has formed in the right lower arm. This is the beginning of a necrotic focus. The cells are for the most part mononuclear phagocytes. $\times 200$.



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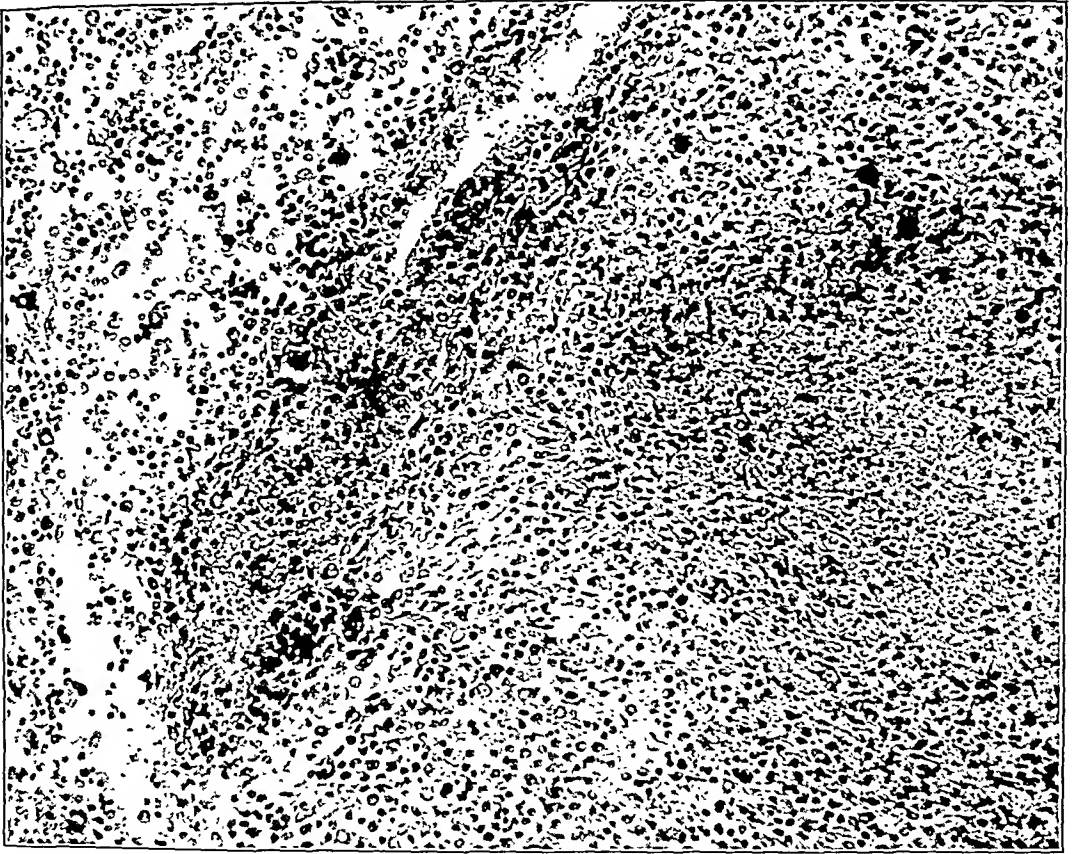


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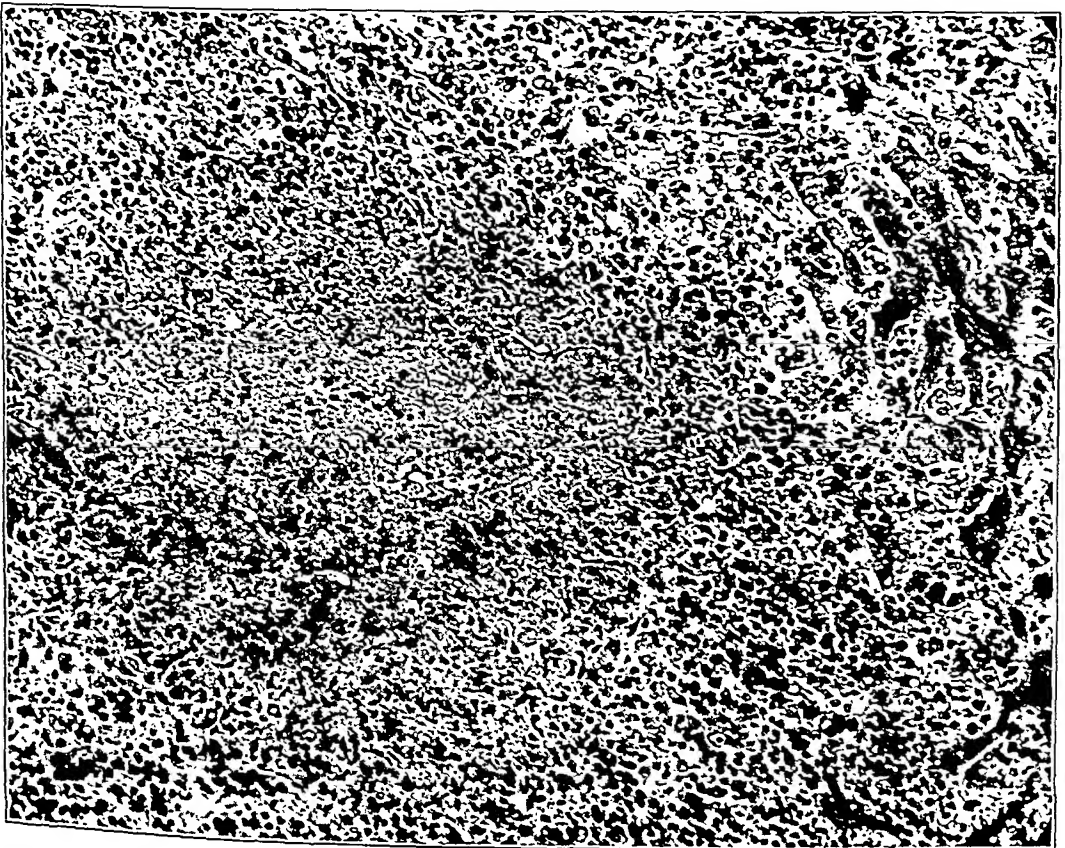
PLATE 59

FIG. 12. Thrombus in a blood vessel in the neighborhood of an acute necrotic focus in a lymph node. $\times 200$.

FIG. 13. Necrotic focus with caseation in the liver. $\times 200$.



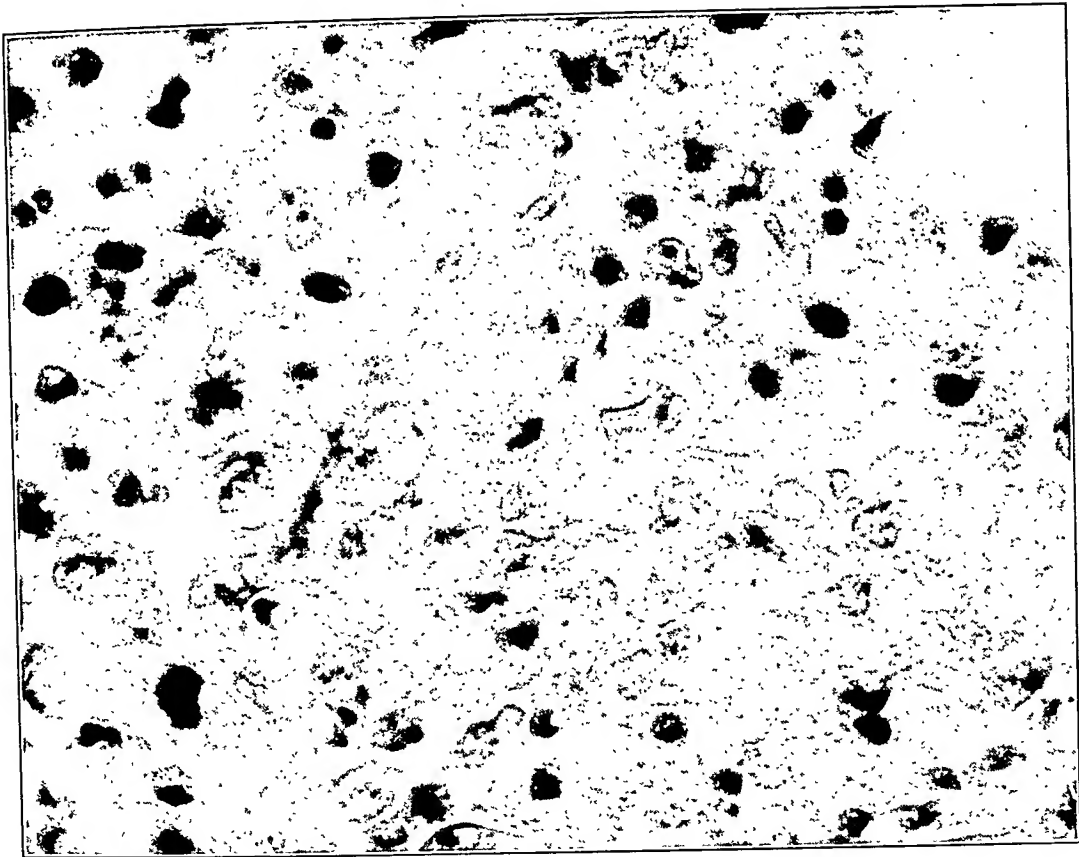
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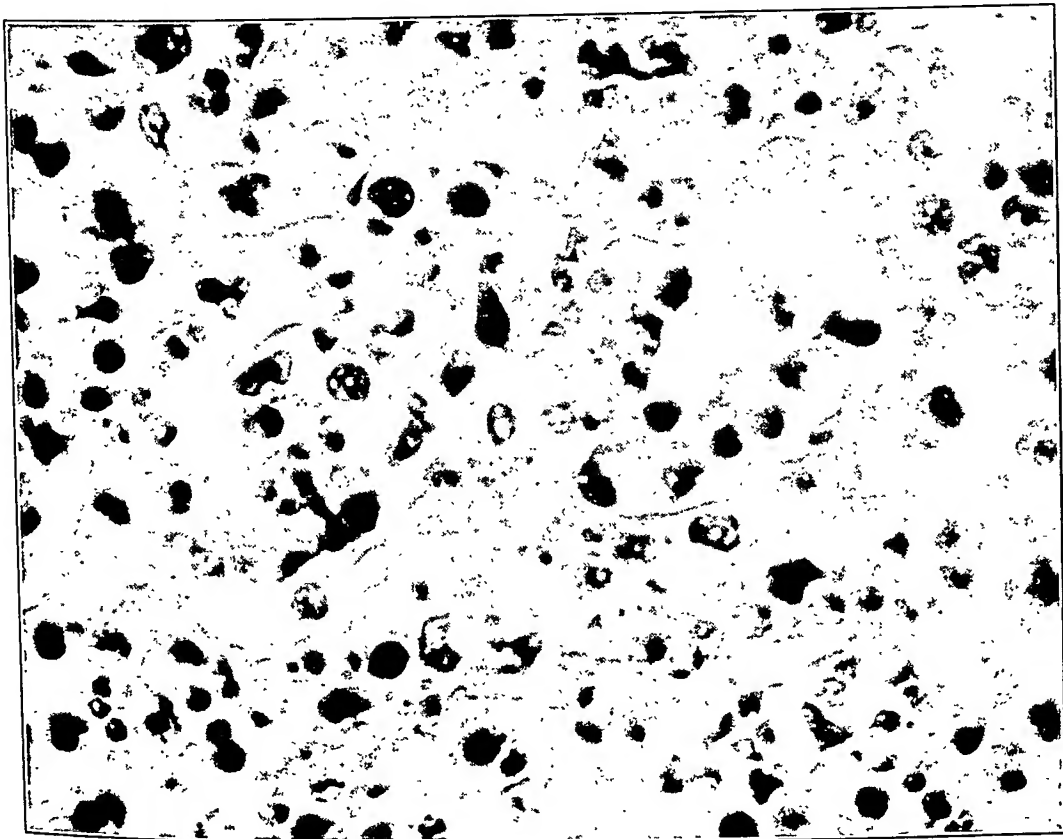
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PLATE 60

- FIG. 14. An early stage in the formation of an inflammatory focus in a lymph sinus. Almost all the cells are of the epithelioid type. Between them is a network of fibrin. $\times 1000$.
- FIG. 15. A beginning focus of necrosis in the parenchyma of a node. Many of the cells are polymorphonuclear leucocytes. Karyorrhexis of nuclei has begun. $\times 1000$.



14



15

STUDIES ON LIPOCHROMES *

I. THE REACTION OF ANIMALS TO THE PRESENCE OF CAROTIN

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The pigments commonly associated with the body fats and usually designated lipochromes have always been of considerable histologic and pathologic interest. However, plant physiologists and biochemists have been the most active in advancing knowledge concerning them, and little attempt has been made to apply the results of their work to human physiologic and pathologic problems. It is known that these pigments occur in relative abundance in certain organs normally, and that upon ingestion in unusual amount they impart a yellow color to the blood and skin. It is also known that in the course of certain metabolic disturbances (diabetes, malnutrition) they accumulate in certain organs to an unusual degree, but it has not been determined what rôle the lipochromes may play under such conditions, or whether they are more than casually associated with them. Most German writers (Oberndorfer¹) believe that they constitute a type of "wear and tear" pigment associated with advancing age and are, therefore, autogenous (endogenous) in origin, but it has been shown by the biochemists that the lipochromes are probably always of exogenous origin and not a product of the metabolic activity of animal cells. The problem has not been subjected to extensive investigation from the pathologic point of view, and it was thought that by experimentation by both chemical and pathologic methods, the subject could be clarified somewhat.

The lipochromes which occur in animals consist chiefly of carotin and xanthophyll. That these are the same substances which occur in plants under similar names was shown by Willstätter and Escher² (1912) for carotin, and by Escher³ (1913) for xanthophyll. They belong to a group designated carotinoids by Palmer.⁴ He prefers the name chromolipoids, and defines them as pigmented substances having in general the solubility of the lipoids, occurring in cells in

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which lipoids are present, and like the phosphatides and sterols, absorbing oxygen readily. They are said to be stained by fat stains.

Carotin has been shown by Palmer and Eckles⁵ to be the pigment present in cow-fat and milk. They identified the carotin of horse blood with the same pigment derived from plants, and Palmer and Kempster⁶ showed that the pigment of egg yolk and of the skin of chickens (xanthophyll) came from their food and was identical with the xanthophyll of yellow corn. Van den Bergh and Snapper,⁷ in 1913, showed that the yellow pigment of the human skin in "Xanthosis Diabetica" was associated with the presence of a similar pigment in the blood, and in later studies by Van den Bergh, Muller, and Broekmeyer,⁸ the further fact that the pigment was the same as the carotin and xanthophyll of the ingested food was brought out.

Two attempts have been made to determine the reaction of the animal organism to these pigments when injected subcutaneously or otherwise. The first, by Wells and Hedenburg,⁹ was to demonstrate a possible toxic action in guinea pigs. These animals were injected intraperitoneally with from 100 mg. to 200 mg. of carotin or chlorinated carotin in olive oil. This had no noticeable effect on the animals, and when killed four days later no lesions were found. The urine was described as of a deep reddish brown color. The intradermal injection of 20 mg. doses produced slight edema only. They concluded that "any such quantities (of carotin) as can ever accumulate in the tissues have no harmful effects." Hess and Meyers¹⁰ injected carotin subcutaneously into infants. They described a yellow coloration of the plasma and urine following this, but no local lesion. The urine became yellower forty-five minutes after the administration of carotin in olive oil by mouth, and the color persisted for six hours. So far as I can find, these are the only reports of similar experiments.

METHODS OF PROCEDURE

The technical difficulties encountered in the preparation of carotin in sufficient quantities for use in animal experiments are many, and after considerable preliminary experimentation the following modification of Escher's method was used.

Fifteen kilograms of carrots were sliced and dried at about 40° C in an oven. The dried carrots were powdered, extracted first with

alcohol, then by continuous extraction with petroleum ether or carbon disulphide. These solutions were evaporated, the first alcohol solution added, and the whole saponified with potassium hydroxide, and again evaporated. The residue was taken up in carbon disulphide, washed several times with 80 per cent alcohol, and spectroscopic examination made. This showed a wide band in the green-blue-violet which obscured the carotin bands; so the solution was evaporated and precipitated by absolute alcohol, the residue taken up in petroleum ether, and washed with 80 per cent alcohol until no further color could be removed. The petroleum ether solution then gave absorption bands at 495-475 uu. and 460-444 uu., corresponding with Willstätter and Stoll's¹¹ 492-476 and 459-445. The petroleum ether solution was evaporated and the orange-red carotin taken up in 20 cc. of pure olive oil. The yield proved to be 26.8 mg. This yield is low because of rapid evaporation in a hood with the fan running, a process which must have oxidized a great deal of pigment which then became soluble in the alcohol. Some was lost in the absolute alcohol precipitation, and by discarding colored solutions which contained also considerable waxy substance, soap, and alkali. But a pure product was desired in order to determine standards for future work.

A more rapid method was used later to secure small amounts for immediate use. Carrots are sliced, boiled with 20 per cent potassium hydroxide in 70 per cent alcohol under a reflux condenser until they are mushy. The mixture is cooled and the alcohol poured off and discarded. (It contains practically no carotin.) The pulp is washed with 95 per cent alcohol in a Büchner funnel, with suction. The partially dried mass of carrots is mashed and mixed with anhydrous sodium sulphite or plaster of Paris, and this mixture is extracted with petroleum ether. A rapid extraction is now possible, limited only by the solubility of carotin in the solvent. Acetone or absolute alcohol may be added to the carrot-petroleum-ether mixture to take up any excess water, in the presence of which the carotin is not separated from the pulp completely. The petroleum ether solution is filtered off, the mass washed with petroleum ether, and the solvent then washed in a separatory funnel with 80 per cent alcohol until no pigment can be removed. The resultant petroleum ether solution responds to the chemical tests for carotin, and if washing has been thorough, the absorption bands in the spectroscope are not obscured

by impurities. Most of the carotin used in the following experiments was produced in this way.

INJECTION OF ANIMALS

A 500 gm. guinea pig was injected intraperitoneally with 2 cc., 3 cc. and 3 cc. of olive oil solution containing 0.67 mg. per cc. of carotin, receiving in all 5.36 mg. over a period of a month. The animal had no noticeable reaction. It was killed ten hours after the last injection. Between the time of the last injection and the killing of the animal two specimens of urine were secured, both only slightly yellow.

A small amount of bright yellow liquid oil remained in the peritoneal cavity, staining the peritoneum everywhere. Small masses of fatty material, yellow in color, contrasting sharply with the normal colorless fat of the mesentery, were present in the omentum and spleen. Most of these nodules were only lightly attached, but several upon the capsules of the liver and spleen were firmly adherent. A few small tubercles were present embedded deeply in the capsule of the liver, and similar, but smaller, pinpoint-sized nodules formed fine lines along the omentum and under surface of the diaphragm, apparently following the course of lymphatics. These lines could be easily seen owing to their yellow color. No nodules were present on the thoracic side of the diaphragm, or in or around the lungs. A mass of yellow fat was firmly attached to the lower (medial) pole of the spleen, but this did not extend for any distance into the substance of the organ, and no pigment was visible within the spleen. The normal retroperitoneal, mesenteric, and all other fat of the guinea pig was colorless as usual. The kidneys, aorta, and other organs excepting the adrenals showed no change, and these latter organs only enlargement. Side by side with the adrenals of a normal guinea pig, those of the carotin-injected animal appeared twice the size. This enlargement was undoubtedly due to the olive oil, rather than the carotin. A control guinea pig, receiving olive oil alone, had also visibly enlarged adrenal glands, with masses of partly encapsulated olive oil in the omentum and mesentery as the only other finding. This enlargement has been noted too often in fat and cholesterol feeding to be commented on here.

Blood, bile, urine and feces were examined from the carotin-injected guinea pig, as follows: Blood, 15 cc. plus 35 cc. 95 per cent

alcohol, was allowed to stand thirty minutes after shaking, until the precipitate had settled. The almost colorless supernatant fluid was poured off, and the precipitate treated with 60 cc. of petroleum ether. No color was extracted. The bile and urine, the latter only slightly yellow, were treated with alcohol, and extracted directly with petroleum ether. No color was extracted. Ten grams of feces from the rectum, colon, and cecum were extracted with petroleum ether after washing with alcohol, and a fair amount of yellow pigment obtained which could not be washed out with water or 80 per cent alcohol and gave the chemical tests for carotin. The animal had, however, been on a diet of hay and oats, with carrots until two weeks before its death.

Sections of spleen, kidney, adrenal and liver were examined unstained or stained with Scharlach R or Nile blue sulfate after fixation in formalin. The spleen section contained a granulomatous nodule attached to the capsule. This showed olive oil in large and small drops surrounded by endothelial cells and fibroblastic tissue. The oil stained red to orange with both stains in most places, but some drops were brownish red from the contained carotin. Many yellow crystals were present in the center of the lesion surrounded partly by neutral fat, and obscured by it when stained by Scharlach R. Considerable pigment was present in the spleen, and remained after thorough washing in acetone and chloroform.

The liver section showed also some adherent granulation tissue, but no lesion inside the capsule. The epithelium of the kidney tubules (collecting and Henle's tubules) contained numerous fine droplets which stained blue with Nile blue sulfate. The adrenal gland contained an immense amount of neutral fat and blue lipid, but it could not be seen that the lipochrome content had increased.

The control guinea pig which received only olive oil intraperitoneally did not have any granulomatous lesions. This oil produces little or no reaction, remaining in the peritoneal cavity as a liquid for several weeks. Small masses of it may be covered by a thin membrane, forming a thin-walled fatty cyst; but it does not produce a granulomatous lesion. Hence, that type of reaction was attributed to the carotin. Its behavior, then, in this animal, was as a foreign body, slowly soluble and absorbable over a long period of time.

Two guinea pigs were injected intraperitoneally each with about 2 mg. of carotin (it is to be remembered that this amount of pigment

would color several liters of petroleum ether a distinct yellow), this time in aqueous suspension. Two hours later specimens of urine were secured. These contained no pigment recognizable as carotin. One of these animals (Guinea pig 2) was killed ten hours after injection. The urine, bile, and blood contained no carotin. Much of the pigment remained unabsorbed in the peritoneal cavity. The second animal (Guinea pig 3) was given a second injection of 10 mg. of carotin in aqueous emulsion with soap, and a third injection a day later of about 200 mg. Four hours and eighteen hours later 4 cc. of blood were taken from the heart. The urine was saved during this time, but no carotin could be extracted from it or from the blood.

Guinea pig 3 was killed three months after the last injection of carotin. There was an adhesion between a loop of intestine and the anterior abdominal wall, formed by scar tissue, in which yellow pigment could be seen. Several yellow nodules were present on the surface of liver and spleen, and on the omentum. The fat of the animal was colorless as usual. Sections of the nodules showed a fibroblastic phagocytic cell proliferation around crystals of pigment which were soluble in chloroform after dehydrating with acetone. They became decolorized when treated with ferric chloride solution for several hours. They did not stain with fat stains. As I have shown elsewhere, these are the only methods which can be depended upon to demonstrate carotin in tissues.

Two other guinea pigs injected with similar amounts of pure carotin showed no reaction to this substance other than that described above. It could not be shown that the carotin appeared in the blood or urine at any time following the injections. Wells and Hedenburg did not identify the brownish material in the urine of the animals which they injected. Hess and Meyers, however, evaporated urine of the injected infants to a small volume, extracted it with petroleum ether, and recovered a pigment which they assumed to be carotin. Carotin is not soluble in urine, and if present would probably be dissolved in a lipid of some sort. It is hardly likely in that case that it could have been extracted directly from untreated, unsaponified urine with petroleum ether. But "urochrome" and bile pigment are also not soluble in petroleum ether. I secured brown urine from a guinea pig (Guinea pig 6) after the injection of 300 mg. of impure carotin. This was secured by the direct extraction of untreated carrot with petroleum ether, and evaporation of the solvent.

Carrots contain, in addition to carotin, xanthophyll and chlorophyll, and brownish and yellowish waxy substances, some of which are removed by saponification, but others only by careful washing and precipitation of the extract. The material injected into Guinea pig 6 contained all these substances, and one or several of them appeared in the urine when collected six hours later. The urine imparted a very faint color to petroleum ether, which, however, could be washed out with 90 per cent methyl alcohol. Its nature was not determined.

Two guinea pigs were fed each with 5 cc. of an olive oil solution of carotin, each receiving 3.35 mg. of carotin. Their urine was saved for the next twenty-four hours, but no carotin appeared in either specimen. Four hours after feeding, 5 cc. of blood were withdrawn from the heart of each animal, and examined for carotin. Both were negative.

A rabbit was injected intravenously on three successive days with small amounts of pure carotin in fine emulsion with soap. The exact amount could not be determined, but well-colored suspensions were used. These produced no noticeable reaction. The urine remained uncolored, and ten hours after the last injection there was no carotin demonstrable in the blood. It was killed ten days later, and no lesions found. The fat, which might possibly be expected to be stained by the pigment, was not noticeably changed in color.

DISCUSSION

Carotin is soluble only in powerful lipid solvents such as carbon disulphide, petroleum ether, ether, and chloroform and in lipoids themselves. It is an unsaturated hydrocarbon, $C_{40}H_{56}$, and is relatively inert chemically. It combines with the halogens and oxygen, and is broken down by strong mineral acids. It would seem that such a substance should behave in the animal body exactly as it has shown itself to behave in these experiments, that is, as a non-toxic, non-diffusible, slowly absorbable substance, forming a characteristic foreign body type of reaction in the tissues. These experiments are not strictly parallel with those of Hess and Meyers. Guinea pigs and rabbits do not normally contain carotin in the blood and none is present in the fat of these animals. It is possible that they may therefore react differently to the ingestion of carotin, though it does

not seem likely that their reaction to this pigment when injected into tissues would differ greatly from that of the human. These and other aspects of this problem will have to be discussed in later papers.

SUMMARY

Carotin, the principal lipochrome found in the animal body, was extracted from carrots and purified. Upon injection intraperitoneally into guinea pigs granulomatous lesions were formed similar to those of familiar foreign body reactions. Carotin did not appear in the blood or urine of guinea pigs after injection or ingestion of relatively large amounts. It produced no effect in a rabbit when injected intravenously.

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STUDIES ON LIPOCHROMES *

II. THE IDENTIFICATION OF CAROTIN, XANTHOPHYLL, AND ASSOCIATED LIPOIDS IN TISSUES

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Early in the course of a study of lipochromes it became evident that a review of the methods by which these substances are identified in tissues was necessary. The common impression among histologists is that these pigments are differentiated from other substances by their positive reaction with the usual fat stains. But it was soon found that they were frequently masked by the fat surrounding them or in which they were dissolved, and that, if they stained at all, they could not be differentiated from other lipid substances which stain an identical color. Also, other crystalline pigments frequently adsorb some of the stains, so that they have the appearance, at least in part, of having been stained by these dyes. The following details were therefore investigated and are described for several reasons. First, a method for positively identifying lipochromes was desirable in order to make future work on these pigments possible. Second, a method for differentiating them from other lipoids in the body is necessary for the study of pigments in general. Third, since lipochrome is almost constantly associated with some other lipid substance, it is desirable to know what that lipid is, and whether it is always the same substance in all places where lipochromes are found. And finally it was thought necessary to clarify the subject of lipid staining in general, as this has always been a puzzle to the writer, and seems to be to most pathologists.

Mallory and Wright ¹ say that free carotin does not stain, but that the fat in which it is dissolved takes the usual fat stains. They give Smith's method with Nile blue sulfate, however, as staining the lipochrome blue. Smith ² states that Nile blue sulfate differentiates fatty acids from neutral fats in the same manner that it appears to differentiate carotinoids from neutral fats, *i. e.*, fatty acids and carotinoid stain blue, and neutral fats red. Dolley and Guthrie ³ assert

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that the lipochromes in plants and animals stain with Sudan III and Scharlach R, even after bleaching. In a second paper,⁴ these writers say that Nile blue sulfate when used as a progressive stain "particularizes the lipochrome first as a deep blue," and stains neutral fat a salmon-pink even in the presence of lipochrome. Palmer and Kempster⁵ report that carotin in frozen sections of carrots and xanthophyll of chicken skin stain blue with Nile blue sulfate. These writers state that lard in which carotin has been dissolved, chicken fat colored with xanthophyll, milk fat (containing carotin) and egg yolk (xanthophyll) are stained salmon-pink by this stain, the pigment presumably not staining characteristically in these solvents, or being masked by the neutral fats present. Hueck⁶ says lipochrome stains blue even after oxidation with hydrogen peroxide. Borst⁷ named the pigment which he found in certain organs lipofuscin because it stained with Sudan III and Scharlach R, and was of the opinion that it must therefore contain some sort of fat substance. According to Staemmler⁸ it is also said to be stained with neutral red, Nile blue sulfate, osmic acid, by the method of Fischler and Smith-Dietrich, at least in part, and is thereby differentiated from melanin. This pigment (lipofuscin) was recognized by Lubarsch⁹ who classified it along with melanin as "wear and tear" pigment (*Abnutzungspigmente*). Staemmler, however, from results with the silver nitrate method of Schreiber and Schneider and the iron cyanide method of Unna concludes that most of those pigments which have been called lipofuscin (lipochrome) are melanin, or forms of melanin. It is obvious that the staining properties of these pigments have not been agreed upon.

EXPERIMENTS IN STAINING

Frozen sections of carrots, unstained, mounted in glycerine, show a surprising amount of yellow pigment within the cells. Most of this is in the form of rectangular crystals, but some fibers resembling fragments of cells are colored yellow. A few cells contain small vacuoles but most appear to be empty, and practically all are devoid of nuclei. When treated with Scharlach R, alcohol-acetone solution, for thirty minutes and washed in 70 per cent alcohol a large number of red droplets appear in the cells, obscuring, or perhaps staining the pigment; but much yellow pigment remains unstained in the cells. Sections of carrots were treated as follows and

stained for from thirty minutes to twelve hours in alcohol-acetone solution of Scharlach R.

1. Sections of carrots which had been extracted with alcohol, then with ether. Considerable color remained. The tissue contained yellowish red pigment, but less in amount than untreated sections. This would not stain; no fat droplets were visible.

2. Sections washed in alcohol-ether, 1:1 solution, then stained with Scharlach R. Some pigment remained, which was not stained.

3. A section stained with Scharlach R in which the pigment was obscured by or stained red by the dye was washed in alcohol-ether solution. All the dye was removed, but most of the pigment, unstained, remained in the tissue.

4. Slices of carrot were placed in olive oil until the oil became well colored. Sections, unstained, showed a few fine yellow undissolved crystals, but most of the pigment had become mixed with the fat which filled most of the cells. The fat droplets had assumed a brownish color. When the section was stained with Scharlach R most of the drops stained red, but some were brownish red, showing that the crystals were surrounded by a thin film of olive oil.

Sections of carrots after treatment in the ways mentioned above were stained with Nile blue sulfate. The stained untreated sections showed nearly everything to be stained but the pigment. The blue color of the cell walls formed a good background for the yellow pigment. A few sections stained overnight showed blue halos around some of the carotin crystals. After treatment with alcohol and ether this color was removed, and the pigment remained unstained. The pigment dissolved in olive oil was masked by this stain, the oil staining salmon-pink, or a brownish pink where the pigment seemed most abundant.

Carrots after treatment by Ciaccio's¹⁰ method (fixed in potassium dichromate, formalin, acetic acid solution 24 hours, treated with 3 per cent potassium dichromate for 3 days, then washed in running water 24 hours) showed no different staining reactions to Nile blue sulfate and Scharlach R. Most of the pigment had not been oxidized by this method, and was still present as yellow crystals. The chromation method of Smith and Mair,¹¹ somewhat similar to the Ciaccio method as to preliminary treatment, but which is followed by a modification of Weigert's hematoxylin method, was not expected to yield any results, as it is supposed to stain only unsatu-

rated fats and fatty acids. Their directions were therefore not followed specifically, but sections after treatment by Ciaccio's method, which should achieve the same result, were stained with Weigert's hematoxylin. It could not be seen that anything but cellulose was stained in the sections.

Frozen sections of adrenal glands and the corpus luteum of an ovary were treated in exactly the same ways as the carrot sections. Details of these procedures may, I think, be omitted. It was found that in the adrenal gland, unstained, the pigment could be seen most prominently in the reticular and inner fascicular zones, where, when stained with Nile blue sulfate, it was more closely associated with a blue-staining material. The outer zones contained the most neutral fat and the least pigment. The pigment of the corpus luteum is more diffuse. (In neither adrenal nor corpus luteum could the pigment be resolved as crystals because of its fine dispersion.) With Nile blue sulfate the pigment is obscured by neutral fat and blue lipoids. Scharlach R, since it stains all lipoids present, and so obscures the pigment, has no differentiating value. After treatment, with fat solvents, the Nile blue sulfate stain brings out the pigments more plainly, as it stains cells and nuclei, and, the fat having been removed, the pigment is more easily visualized.

Preliminary studies with neutral red and safranin showed that these stains had no particular differential qualities when applied to this pigment. Osmic acid was not tried, as it was thought that no differentiation from fats could be made even if this substance were oxidized. Wells¹² says carotin is usually but not constantly affected by osmic acid; Herxheimer¹³ says it always is.

Hueck, and Dolley and Guthrie state that after oxidation with hydrogen peroxide or ferric chloride, carotin stains with Nile blue sulfate. Both these methods were tried. Frozen sections of carrots, adrenal gland and corpus luteum were treated with 3 per cent hydrogen peroxide in a covered dish, and with a strong solution of ferric chloride on slides. The yellow pigment was oxidized in the course of a few days in hydrogen peroxide, and in a few hours by ferric chloride. It was still visible in reduced light in the carrot sections as pale crystals, but could not be found in the adrenal or corpus luteum in unstained sections. When stained with Nile blue sulfate, the blue of the pigment, if it stained at all, could not be differentiated from cell fragments, cellulose, nuclear granules, etc.,

in the carrot section, and could not be differentiated from other blue-staining material in the adrenal and corpus luteum sections. This procedure also oxidizes some neutral fat which then stains blue also, making the picture more confused than ever. However, the fading of the crystals or the disappearance of amorphous granules when treated with ferric chloride is evidence of the nature of the pigment. Hematoidin and hemosiderin are not acted upon by this substance, and melanin, some of which may be oxidized by prolonged treatment with ferric chloride, first becomes black. This was checked in the adrenal section which contained medullary pigment. The melanin was plainly visible long after the lipochrome had disappeared.

The Molisch method ¹⁴ of demonstrating carotinoids in plants has never been applied to animal tissues, according to Palmer. This consists of placing the plant (leaves, petals, etc.) in a 20 per cent solution of potassium hydroxide in 40 per cent alcohol, allowing it to stand in the dark for varying periods of time, and observing the crystals which are then visible under the microscope. When animal tissues are treated by such a strong solution of alkali they dissolve before crystallization of the carotin can take place. A 10 per cent solution of potassium hydroxide in 70 per cent alcohol was therefore tried, but the cells of the section (adrenal gland) were pretty well digested in 5 minutes. A 10 per cent solution of potassium hydroxide in 70 per cent alcohol plus an equal amount of 4 per cent formaldehyde was found to dissolve cells slowly and to produce the best results, which, however, were not particularly good. A section of adrenal gland in which a dispersed yellow substance was visible was treated for forty minutes in this solution. After washing in water and mounting in glycerine the yellow material was found to be denser in some places, and absent in others, having apparently been released from its former association with fat. Drying the section before mounting brought out the crystalline character of the pigment. When a section of heart muscle was treated the same way the result was inconclusive, as much amorphous pigment remained scattered in the muscle fibers. While this method would serve to reveal lipochrome when it is the only pigment present, and to differentiate carotin from chlorophyll in plant tissues, it could not be used to separate pigments of two or more kinds in animal tissues.

Smith ² gives the following staining reactions of various lipoids

with Nile blue sulfate: neutral fats, red; cholesterol and esters, pale red or light blue; phospholipins, pale blue; soaps and fatty acids, deep blue; carotin, deep blue; melanin, yellow-green (not stained). This is the dye now most often used to differentiate various lipoids in tissues, but from the preliminary observations recorded above it seemed that this wide range of differential colors could not be depended upon with certainty. If, however, this dye could be found actually to distinguish specific lipoids, one of our questions, namely, the kind of lipoid with which lipochrome is associated, might be answered by its use. Accordingly the following experiments were tried:

1. A rabbit was injected intraperitoneally with pure cholesterol in as heavy a suspension as could be made with water, repeated doses being given over a period of a month until about 2 gm. of cholesterol had been injected. Granulomatous lesions were present in the omentum, upon the spleen and liver, and in the mesentery when the animal was killed. Frozen sections of these lesions showed a typical foreign body reaction around crystals of cholesterol. When stained with Nile blue sulfate many of the giant cells present were seen to contain a deep blue liquid material. The crystals did not stain. Many smaller phagocytic cells contained blue or purple to purplish red droplets. These had a blue center, and a purple or reddish rim. Whether the cholesterol in the giant cells immediately surrounding the crystals had been changed before or at the time of liquifaction or whether it was simply dissolved in the cytoplasm of these cells could only be guessed at, but it stained deep blue, not pale red or light blue. As it changed color in the smaller cells at a distance from the crystals, it apparently had become changed either by esterification or by admixture with neutral fat. As the centers of these droplets were blue and the peripheries red, it seems likely that the latter occurred, particularly in view of the results of the second experiment.

2. Cholesterol was mixed with an excess of acetic anhydride and this boiled for two and a half hours under a reflux condenser. After adding water and cooling, the precipitate was taken up in petroleum ether, from which long needles precipitated upon standing. These were washed with water, then 95 per cent alcohol several times, and dried. The melting point was about 114° C (M. P. of cholesterol $147-148^{\circ}$ C). The cholesteryl acetate was injected (about 200 mg.)

in aqueous emulsion into a guinea pig, intraperitoneally. Eleven days later when the animal was killed small nodules were present which contained crystals of the cholesteryl ester. Small giant cells around these crystals contained a deep blue material when stained with Nile blue sulfate and the edges of many of the crystals where they were apparently undergoing solution were blue. Smears from the peritoneum contained considerable liquid blue-retaining material. A few pink globules were present in and outside of cells. A section containing normal neutral fat, used as a control, stained pink.

3. Similar experiments were made with oleic acid, and boiled oleic acid plus cholesterol. The latter was possibly cholesteryl oleate mixed with cholesterol. Their staining reactions were the same as with cholesterol and cholesteryl acetate. Soap and lecithin, upon injection, also stain blue.

4. The behaviour of Nile blue sulfate in certain solutions was determined. It was found that it acts as an indicator, and is blue when neutral or acid, and purplish red to orange-red when alkaline. According to Smith it is a mixture of a strongly basic oxazine which unites with fatty acids to form blue soaps, and a weakly basic oxazone which dissolves in neutral fat, and is orange-red in color. That it does not behave as an indicator in animal tissues is shown by this reversal of color reactions. In vitro the dye behaves toward fatty acids and soaps as in the body. A soap solution even though ordinarily alkaline is colored blue, as well as fatty acids (oleic, stearic); but neutral fats are not colored red. Neutral olive oil dissolves the crystalline dye with great difficulty, turning blue. Two drops of 0.1 normal sodium hydroxide turn it reddish brown, and two drops of oleic acid turn it blue again. Pure tristearin stains blue in vitro. However, when in a thin film on a glass slide, the neutral fat mixtures with the dye assume a purple to red color. In the colorimeter both the fat and soap mixtures show two colors, namely, blue and red. The red is removed by adsorption with filter paper in watery solution and from soap and fatty acid mixtures.

There seem to be therefore both in vitro and in vivo, only two reactions to this dye, namely, a selective reaction of soaps, fatty acids, cholesterol and its esters, and lecithin, with the blue, and a similar physical or chemical affinity of neutral fats for the red. It seems likely that purples, reddish blue, and other slight changes in color represent only admixtures of the blue-staining substances with

neutral fat, and not specific lipoids. This dye would not therefore be of great use in differentiating the type of lipoid with which lipochromes are associated, beyond ruling out neutral fat.

It was determined by means of a Nicol's prism attached to the microscope that carotin in carrots and adrenal gland, and xanthophyll, the lipochrome of chicken skin, are not doubly refractive (anisotropic). In carrot sections some crystals are associated with anisotropic fat, as determined by their double refractiveness in stained sections, but numerous small droplets or irregular transparent masses are present which are doubly refractive and which are not associated with carotin crystals. Furthermore, when all the fat is dissolved out with ether, the carotin which remains (being less readily soluble) is not anisotropic. This doubly refractive lipoid of carrots with which the carotin is sometimes associated is presumably phytosterol.

It was more difficult to distinguish the carotin from the abundant anisotropic fat of adrenal gland. Lipoids cannot be dissolved out without extracting most of the pigment also. But what pigment remained in the zona fascicularis after such treatment was not doubly refractive. In chicken skin, where recognizable as xanthophyll, this pigment is not doubly refractive. In this organ the pigment occurs as crystals in the outermost layers of the corneum. Deeper in the tissue it is more widely dispersed, and is associated with fatty substances which stain purplish red with Nile blue sulfate. The corneum itself is impregnated with a lipoid which stains with Scharlach R, and becomes pink in Nile blue sulfate preparations. In the Malpighian layer, pigment could not be differentiated from blue-to-purple-staining fat. A thin luminous line of anisotropic substance is present where the cells of the Malpighian layer and the keratinized outer cells meet. This is the zone which stains purple with Nile blue sulfate. It probably contains a mixture of cholesterol and neutral fat. The anisotropism shown in this layer disappears on warming the section, a phenomenon supposed to characterize cholesterol and its esters.

Palmer and Kempster found xanthophyll to stain blue in the deeper layers of chicken skin. I could not be certain that the blue-or purple-staining material in the Malpighian layers was lipochrome. In the deeper layers, this substance is so finely dispersed that it could not be recognized by the methods described above. Only in

the outer scales of the corneum could it be resolved as a distinctive substance. And here it did not stain at all, and was not doubly refractive.

SUMMARY AND CONCLUSIONS

By the use of the usual fat stains on frozen sections of carrots, adrenal gland, corpus luteum, and chicken skin after treatment with fat solvents and other reagents, and on tissues removed from animals after injection of cholesterol, cholesterol esters, fatty acids, soap, and lecithin, and by the use of other reagents which might be expected to distinguish lipochromes from other substances in tissues, an effort was made to differentiate carotin, xanthophyll, cholesterol and other lipoids from one another. This has not been entirely successful, but the results justify the following conclusions:

1. Carotin and xanthophyll, the two common lipochromes, do not stain by any of the fat stains ordinarily employed to differentiate them.

2. When associated with a lipid which does stain, this lipid takes a blue stain with Nile blue sulfate, and may therefore be cholesterol, cholesterol ester, soap, fatty acid, or lecithin.

3. Because the substance with which the lipochrome is associated is usually anisotropic, and carotin and xanthophyll are not, this substance is assumed to be cholesterol or an ester of cholesterol.

4. When a pigment is present in tissues associated with a substance which takes a specific stain for fat, this pigment may be assumed to be a lipochrome, but this is not invariably so.

5. Yellow pigment which tends to coalesce when treated with weak alcoholic potash and formalin, or which crystallizes in the tissue after such treatment, is probably lipochrome.

6. Pigment which disappears or loses its color after treatment with a strong solution of ferric chloride (or other oxidizing agent), if not continued over too long a time, may be assumed to be lipochrome.

7. Pigment which is soluble in the usual fat solvents (ether, petroleum ether, chloroform, etc.) is probably lipochrome, but this treatment must be prolonged, and used after dehydration of the tissue with alcohol or acetone to be effective and of differential value.

8. Nile blue sulfate differentiates only neutral fats from all other lipoids, neutral fat staining red, and all other lipoids, which stain, blue. Lecithin, which takes a somewhat lighter stain than the other

blue-staining lipoids, may possibly be differentiated from them but careful timing of the staining process is necessary.

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ANGIOMA RACEMOSUM VENOSUM — REPORT OF A CASE *

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The explanation for the sudden death of a young pregnant woman was found at necropsy to be an intraventricular hemorrhage from the rupture of a vessel of an angioma racemosum venosum — a rare abnormality of the vascular system of the brain.

The rareness of the lesion, the unusual circumstances and the opportunity of presenting a histologic study of the lesion prompted the following report.

REPORT OF CASE

Clinical History: The patient, a white female, aged 23 years, was in about the eighth month of her first pregnancy and apparently in excellent condition. During the two months preceding admission the patient had fallen five separate times while walking. Preceding each fall, the patient experienced a sudden loss of strength in the legs. The last fall occurred five days before admission. During the attacks there was no failure of vision, no pain, no dizziness and no loss of consciousness. Strength returned in about five minutes, and the patient was always able to resume the walk. Nothing in the past history appeared to be related to the present condition.

On the morning of admission the patient felt in her usual good health. Shortly after arising and while at the toilet the patient suddenly cried out that she was blind and then fell unconscious. Two hours later the patient was brought to the New Haven Hospital and died there shortly after admission. The patient was unconscious when admitted. The pulse rate was 76 per minute; the blood pressure, systolic 100, diastolic 60; the temperature 99° F; and the respirations were stertorous and slow (8 per minute). The body was cold and cyanosis was extreme. There was no subcutaneous edema. Numerous moist rales were heard in the lungs. There were no convulsions. The urine examination showed a very faint trace of albumen and a few hyaline casts.

Necropsy Report: The post-mortem examination was made three hours after death. The findings explaining the cause of death are confined to the brain.

The blood vessels of the superior and lateral surfaces of the brain are distended with blood and the subarachnoid space contains enough blood-tinged fluid to give the surfaces a bright pink color. The basal cisterns are distended with blood, which was aspirated

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from the ventricles before the brain was opened. After injection with and fixation in 10 per cent formalin the sections of the brain show the ventricles to be filled with clotted blood. A single, non-encapsulated, vascular, spongy tumor occupies the greater part of the right occipital lobe (Fig. 1). The accompanying pictures show the exact location. The tumor is made up of thin-walled vessels of various caliber surrounded by a translucent gray-white tissue. The largest vessel is 7 mm. in diameter and is located in the posterior medial portion of the tumor. This vessel is connected with a number of the smaller vessels of the tumor and also with two thin-walled pial vessels. The latter vessels are approximately 2 mm. in diameter. The pial vessels adjacent to the tumor are larger than the pial vessels of the opposite occipital lobe. The connection of the smaller vessels of the tumor and of the adjacent pial vessels to the large vessel is demonstrated by injecting a gelatin mass into the large vessel and watching the smaller vessels fill. With the aid of a lens, focal thickenings of the vascular walls are seen. No thrombosed vessels are found. Several small calcareous deposits are felt in the vessel walls. In the posterior horn of the right lateral ventricle the vessels of the tumor have pushed up the ependymal lining and one vessel is seen with a tear about 1 mm. in length which is coated over with recently clotted blood.

Microscopic Examination: Sections from the lesion were stained with Mallory's phosphotungstic acid hematoxylin, hematoxylin-eosin, Weigert and Verhoeff's elastic tissue methods and Perdrau's connective tissue method.

The lesion is composed of a large number of blood vessels of varying size and shape. The largest vessel (Fig. 2) measures 11 mm. in its greatest diameter while many other vessels are from 3 to 7 mm. in diameter. The vessel walls vary greatly in thickness. The greater portion of all of the vessels resemble medium-sized or small veins. There are, however, numerous fusiform or nodular thickenings of the intima occasionally, but most often of the media, which, in Fig. 2, are seen to occur gradually or sharply at irregular intervals along the otherwise thin-walled vein. The phosphotungstic acid hematoxylin stains bring out the fact that myoglia fibrils (Fig. 3) predominate in the thickenings of the media although collagen, fibroglia and elastic fibrils are also seen.

The intima is composed of a single layer of endothelial cells.

Scattered, irregular subintimal thickenings are composed of loosely arranged cells resembling young connective tissue cells (Fig. 5). An incomplete internal elastic layer is found in the thickened portions of several large vessels (Fig. 4), although small fragments of elastic tissue are seen in other vessels. The media of several of the large veins contains small calcium deposits. The local nodular and the fusiform thickening of the media, projecting into the lumen, are described above. The adventitia is thin. No cells of mesodermal origin are found between the vessels of the lesion. The greater portion of the tissue between and at the periphery of the vessels is composed of dense neuroglia fibrils and degenerated brain tissue.

No evidence of hematopoiesis is seen in the sections but smears from the blood of the tumor were not made.

DISCUSSION

The lesion described is considered to be a congenital anomaly of the vascular system although it is named angioma racemosum venosum. Because of the variations in nomenclature and the absence of verification by histologic methods, difficulty has been experienced in selecting cases from the literature. There are but three recorded instances of angioma racemosum venosum which were verified by histologic study.* Thirteen reported cases seen at operation and one at necropsy presented the typical gross appearance of this type of vascular abnormality — a collection of non-pulsating, tortuous, thin-walled vessels distended with dark blue blood — but no microscopic examination was made. Another example of the rarity of the lesion is the fact that among 1500 verified cases of intracranial tumors Dr. Harvey Cushing has seen at operation only five examples of angioma racemosum venosum and in none of these cases was it possible to remove tissue for histologic study.

The lesion has not been diagnosed clinically. It has been usually seen at exploratory cranial operations and recognized by the previously mentioned characteristics. In the several cases examined at necropsy there has been noted a wedge-shaped collection of veins of various caliber which projected into the cerebral tissue for a varying distance from the superficial collection of veins. The histologic studies in the three reported cases showed changes in the vessel walls similar to those described in this report.

* See Bibliography: Dürck, E. Herzog, E. Therman.

No previous report has been found of rupture of a vein of an angioma racemosum venosum into the brain or ventricle with subsequent hemorrhage and death.

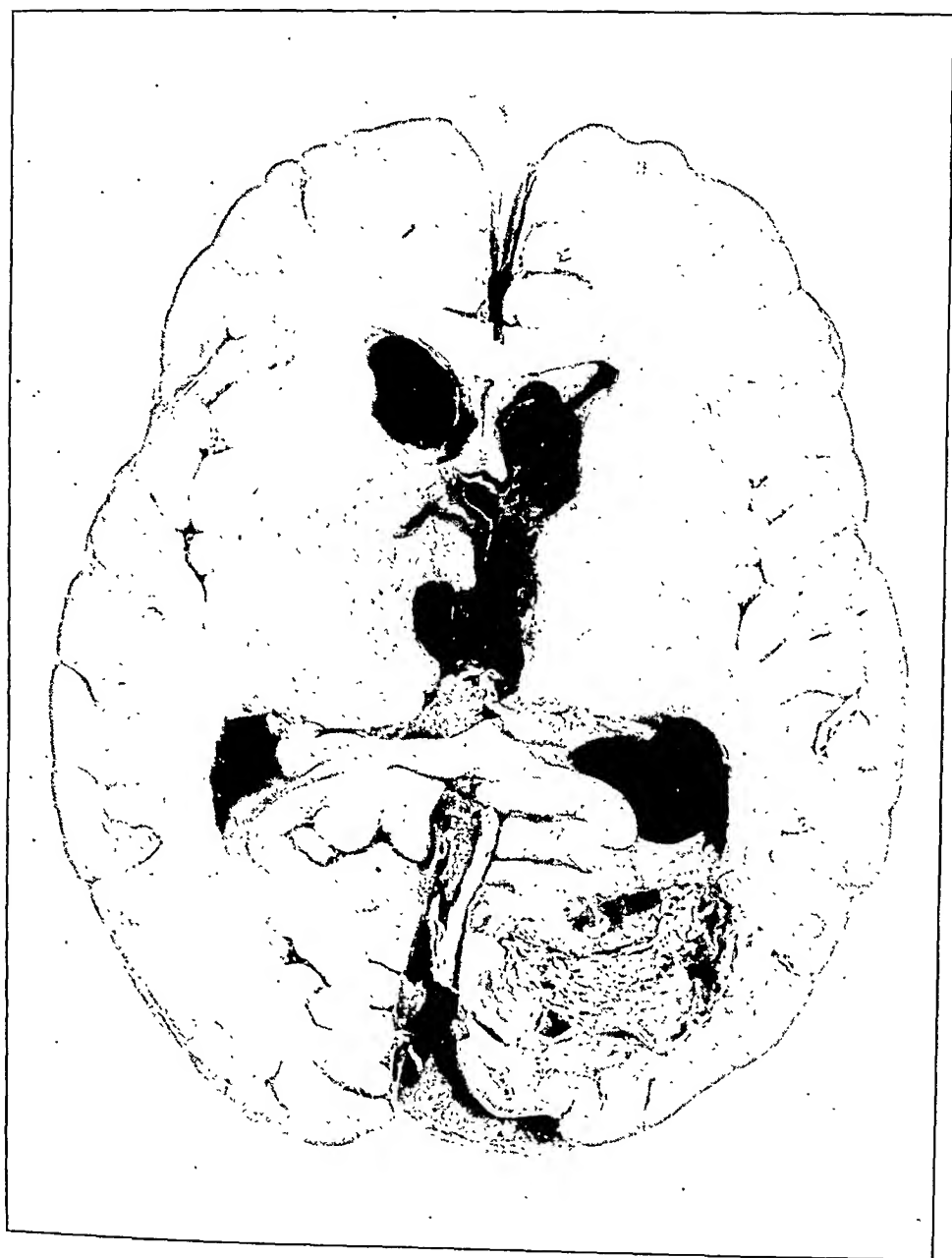
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DESCRIPTION OF PLATES

PLATE 61

FIG. 1. Abnormal collection of vessels in the right occipital lobe with rupture and hemorrhage into the lateral ventricles.

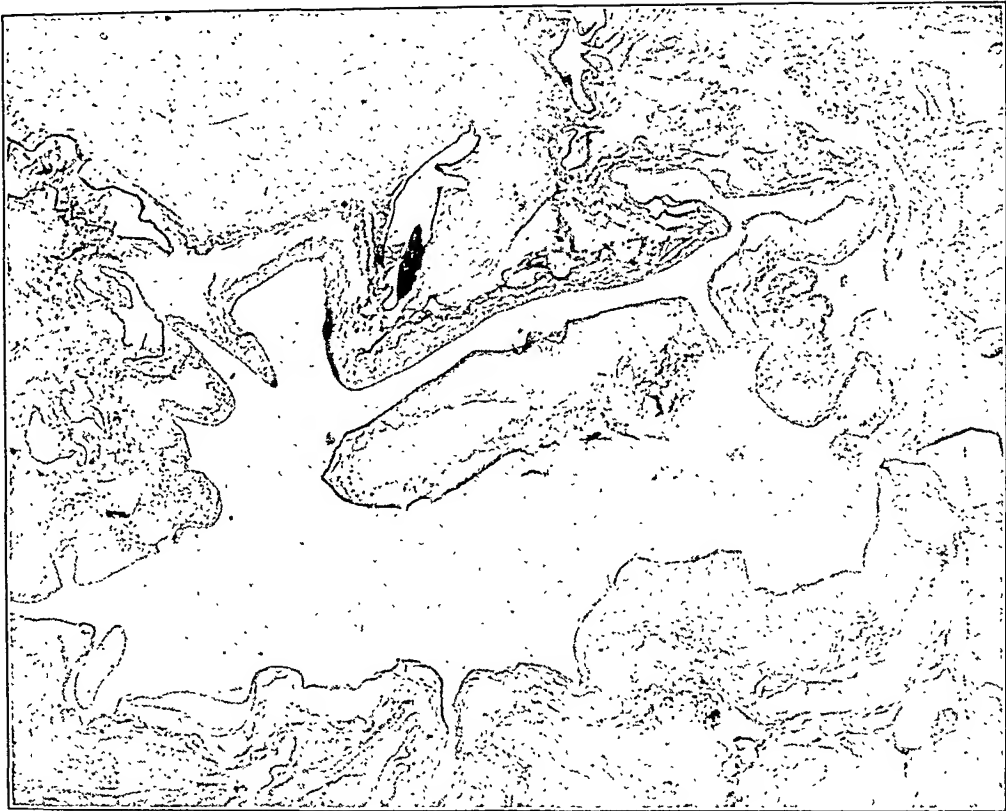


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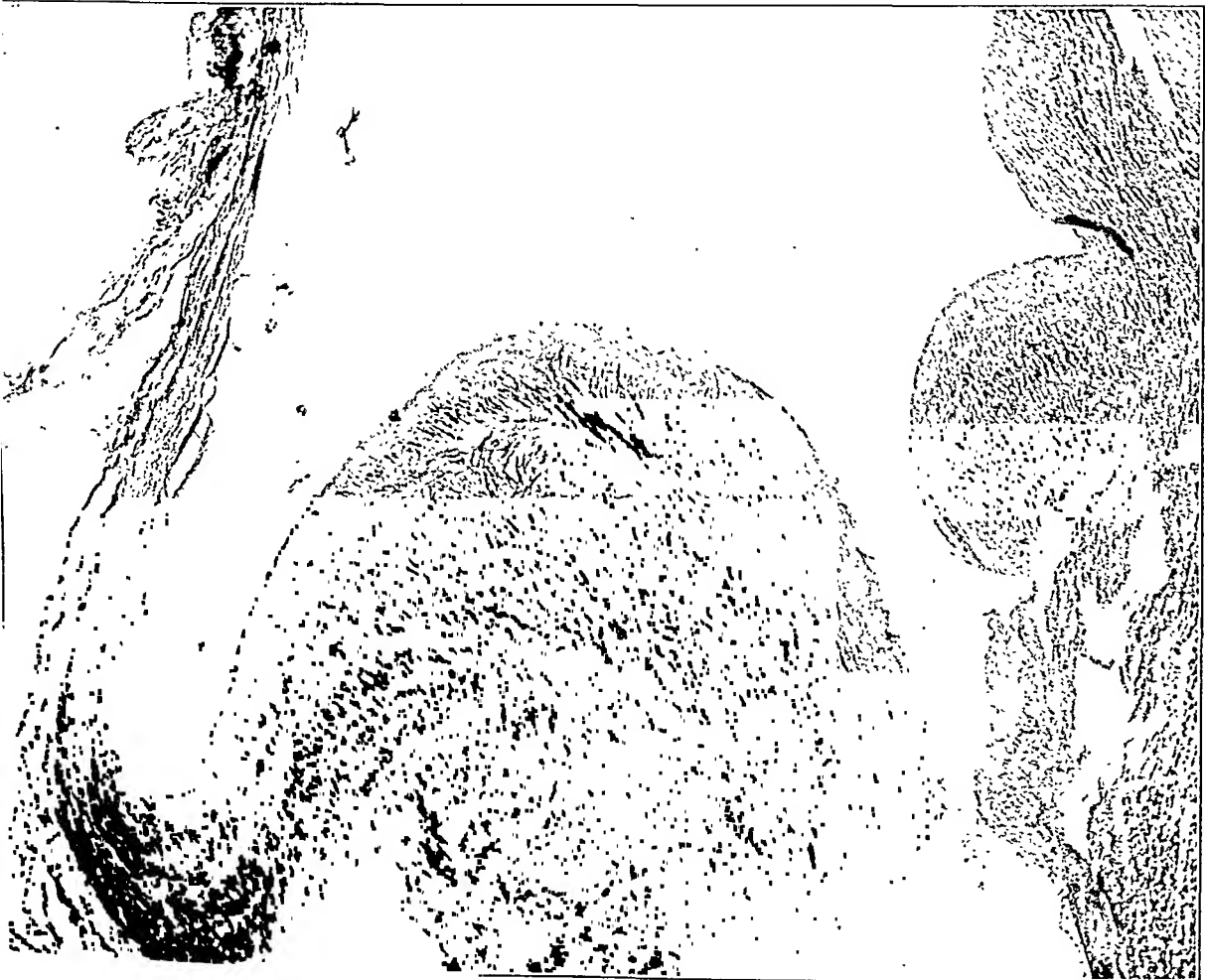
PLATE 62

FIG. 2. Cross-section of large, irregular vessel seen in posterior medial portion of Fig. 1. Note the numerous subintimal and medial thickenings. H. and E. $\times 10$.

FIG. 3. Leiomyomatous thickening of the media stained with phosphotungstic acid hematoxylin to show the myoglia fibers. $\times 80$.



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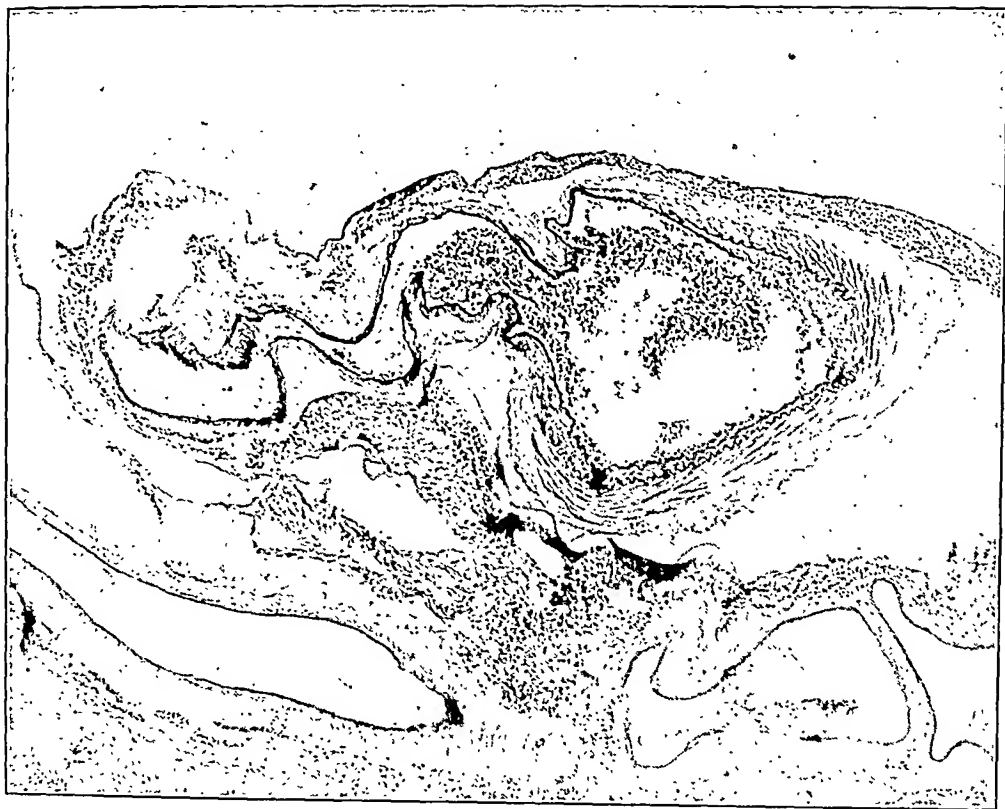


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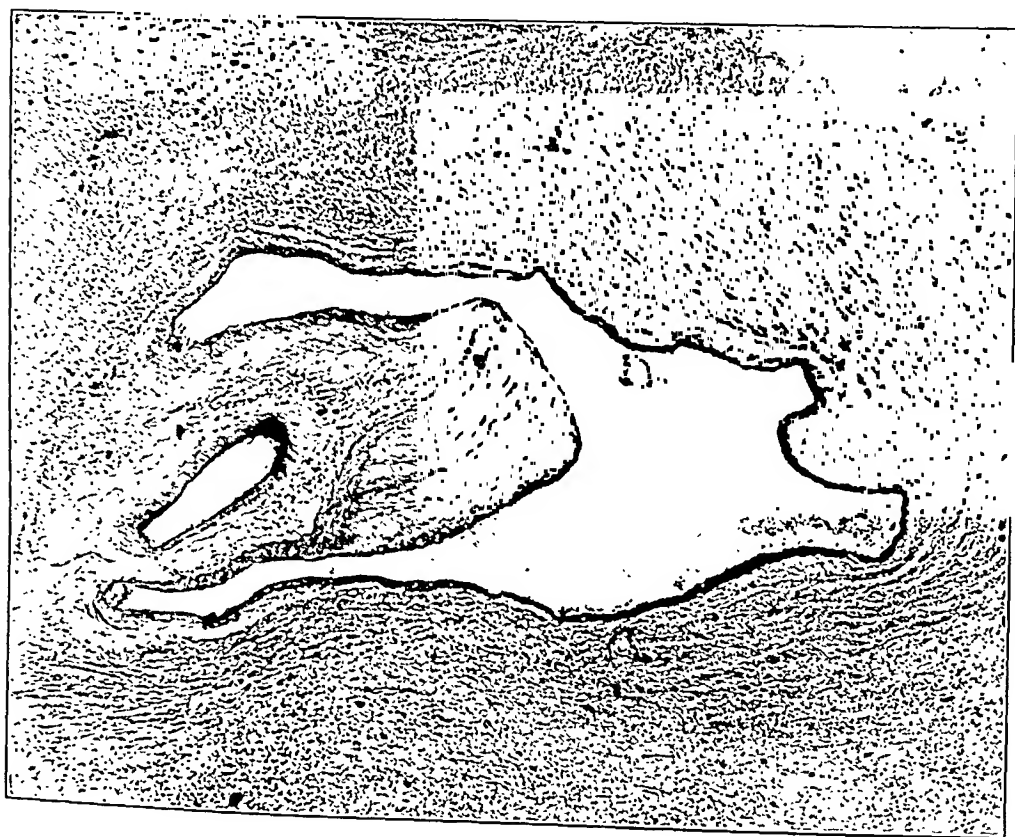
PLATE 63

FIG. 4. Irregularly shaped vessel stained by Weigert's elastic tissue method to show the incomplete internal elastic lamellae. $\times 20$.

FIG. 5. Nodular subintimal thickening surrounded by degenerated brain tissue. Weigert's elastic tissue stain. $\times 30$.



4



5

SPONTANEOUS RUPTURE OF THE HEART *

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Attention has been called to this subject by the recent review of the literature by Krumbhaar and Crowell,¹ and de la Chappelle.² The incompleteness of the publications reporting incidences of spontaneous rupture of the heart is emphasized. In the pathology service of the New Haven Hospital there have been three cases of spontaneous rupture of the heart which seem to be of sufficient importance to present in some detail.

CASE I. *Clinical History.* The patient, a white male, aged 35 years, came to the New Haven Dispensary on July 14, 1924, complaining of lameness, pain, numbness, coldness and prickling sensations occurring in the left lower leg and foot following a short walk. Walking could be resumed after a rest but the symptoms soon returned. The patient had had a drawing sensation over the precordium during the previous year, but otherwise was in excellent condition. The examination showed that the arteries of both legs and feet pulsated. There was, however, a faint erythema of the skin of the left big toe which was not present in the skin of the rest of the foot. The urine examination showed a trace of sugar which disappeared with dietary regulations. Rest was prescribed but there was no relief and the patient came into the hospital for observation. A periarterial sympathectomy was advised but refused and the patient was discharged. During the next month the original symptoms became more severe, and the patient reëntered the hospital. A periarterial sympathectomy of the left leg was successfully done. The patient's condition improved so that he was discharged on October 1, 1924. During the next three weeks the previous condition became worse and on return to the dispensary the patient was advised to reënter the hospital to have his big toe amputated. At this time the big toe of the left foot was dry and black. There was a line of demarcation with a reddened edematous skin on the dorsum. The other toes appeared normal. The dorsalis pedis and posterior tibial arteries could not be felt to pulsate. Five days after this admission an amputation of the left big toe was made. In the post-operative course the wound became gangrenous and did not heal. It was then decided to amputate at the middle of the thigh. On December 25, 1924, the patient was transferred to the medical service for treatment of the diabetes. It was found that on ten units of iletin per day the urine was sugar free. At this time the patient complained of pain in the left upper chest and of shortness of breath. A pericardial friction rub was heard which was present for a day and a half. Following this the patient had a temperature ranging

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from 100° to 102° F. The patient complained of considerable pain at the stump of the left thigh, and an excision of the sciatic nerve was made. Histologic examination of the nerve and surrounding tissue was negative. Following this operation symptomatic relief was obtained. The patient had considerable pain in his left shoulder, especially following pressure in the region of the apex of the heart. This pain was persistent in the left supraclavicular portion but also radiated to the upper portion of the left arm. The patient's condition was apparently improving when suddenly, on March 9, while sitting up, he fell forward and began to gasp. He was very cyanotic, the pulse was not felt, and the heart sounds were irregular with a rate of 40 per minute and a large auriculoventricular interval. The patient died shortly afterward. A clinical diagnosis of coronary occlusion as a terminal event was made.

Necropsy Report: The chief findings of the post-mortem examination are in the cardiovascular system and these only will be described. The pericardial sac is distended by about 600 gm. of fluid and clotted blood. The clotted blood is easily pulled off every part of the visceral pericardium except over the posterior part of the apical saccular dilatation. Here the pericardial adhesions are firm and the clot is lightly adherent to the junction of the adhesions and myocardium and to a small three-cornered rupture of the aneurysmal dilatation. The openings of the coronary arteries are patent. A dissection of the main branches of the right and left coronary arteries shows that before they divide their wall is raised here and there with small light yellow plaques which encroach only to a small extent upon the lumina. As the anterior descending branches of both arteries are dissected the luminae become smaller. A cross-section of the anterior descending branch of the left coronary artery 2 cm. from the beginning at this area shows an almost completely obliterated lumen with only a small, pin-point drop of blood exuding from the central portion of the artery. As the artery is followed down it is seen that this same condition holds to the apex. The descending branch of the right coronary artery shows a similar picture but even more extensive in that there is no lumen to be seen in any portion shortly after its origin. At the apex there is a sudden transition from the thick muscle wall to a thin fibrous tissue wall from 1 to 2 mm. in thickness which bulges out to form an oval-shaped dilatation measuring about 6 cm. in any diameter. This large aneurysmal dilatation is completely filled and distended with blood clots of varying ages. The center of this mass is made up of gray-white, very friable, clotted material which extends only a short distance into the cavity of the left ventricle. The parietal pericardium is firmly adherent to

the apex wall. On cross-section it is difficult to distinguish between the adherent pericardium and the wall of this dilated portion of the ventricle itself. Directly above this dilated area the endocardium of the left ventricle is pearl-white in color. The myocardium of the lower and middle part of the left ventricle is streaked with or almost completely composed of gray-white, dense, fibrous tissue. The wall of the right ventricle averages 6 to 7 mm. in thickness. The left ventricle wall measures 18 to 19 mm. in its upper half and 12 to 13 mm. in its lower portion just before it sharply changes to the wall of the aneurysmal dilatation. The sinuses of Valsalva are normal. The aorta, renal, and splenic arteries show considerable sclerotic change.

Microscopic Examination: Histologic studies are made of the myocardium, wall of the aneurysmal sac, and of the coronary vessels. There is complete occlusion of the coronary vessels supplying the lower half of the left ventricle, and infarction, mural thrombus formation, and a healing process by which a new wall at the apex is formed. Compensatory hypertrophy of the remaining cardiac fibers is present. The myocardium of the left ventricle is badly damaged. Dense masses of fibrous tissue and an increase of interstitial tissue represent the healed process. Necrotic cardiac fibers with opaque cytoplasm and pyknotic or absent nuclei represent the more recent process. Adherent organized or organizing thrombi with fresh thrombotic deposits are seen at the apex. The undamaged cardiac fibers are hypertrophied and their nuclei are peculiar in their increase in size and irregularity. Scattered through the myocardium and in the loose subepicardial tissue, there are large deposits of mononuclear and plasma cells about the vessel sheaths. The wall of the saccular dilatation is less than 1 mm. in thickness in places. It is made up of a very loose connective tissue which is infiltrated with mononuclear cells. Near the point of rupture there are extravasations of red blood cells into the recently organized wall. The descending branches of both coronary vessels are completely occluded by a dense, acellular, light pink tissue, the center of which contains several thin-walled capillaries. In the media of the left branch there are large calcium deposits.

Anatomic Diagnoses: Primary. Generalized arteriosclerosis with special involvement of: (a) posterior tibial and dorsalis pedis arteries; old operations (amputation of toe and left leg for gangrene; periar-

terial sympathectomy; resection of sciatic nerve, left); (b) coronary arteries with occlusion of descending branches, right and left; healed infarct of left ventricle; aneurysmal dilatation of infarcted area of left ventricle and rupture of its wall; hemopericardium; hypertrophy and dilatation of the left side of the heart; congestion of the viscera.

CASE II. *Clinical History.* A white man, about 60 years of age, collapsed on a street car and died en route to the hospital. No clinical history was obtained.

Necropsy Report: The post-mortem examination is made shortly after death. The principal findings are in the cardiovascular system. The pericardial sac is distended with about 100 cc. of fluid blood. The visceral pericardium is covered by an elastic, non-adherent, recent blood clot about 1 cm. in thickness except over an area 3 cm. in diameter on the anterior surface of the left ventricle where the pericardial layers are bound by easily separated fibrinous adhesions. The heart is enlarged and weighs 650 gm. There is a ragged tear 5 mm. in length on the anterior surface of the middle of the left ventricle 1 cm. to the left of the septum. This extends into the left ventricle and is filled with a friable clot which is continuous with a thrombus adherent to the inner ventricle wall about the ruptured myocardium. The subepicardial fat is thick in all areas. The myocardium about the point of rupture and at the apex of the left ventricle is thin, averaging 4 mm., and contains considerable gray-white scar tissue. Elsewhere the myocardium of the left ventricle averages 14 mm. in thickness. The anterior descending branch of the left coronary artery is almost entirely occluded 4 cm. from its origin except for a small pin-point lumen which is filled with a bright red clot.

Microscopic Examination: Histologic sections about the point of rupture show a fibrinous exudate over the pericardium, a recent mural thrombus attached to the endocardium, and necrosis of the cardiac fibers with an extravasation of red blood cells and an infiltration of polymorphonuclear leucocytes between the necrotic fibers. The margin of the rupture is coated with a fibrin network and red blood cells. There are other areas of fibrosis with hypertrophy of the remaining fibers at the apex. The coronary vessels show varying degrees of occlusion from the only slightly raised intimal thickening

to almost complete occlusion. The anterior descending branch of the left coronary artery has only a small slit left as a lumen. The intima of this vessel is thickened by light pink-staining, acellular tissue and an amorphous material containing many crystal spaces as well as calcium deposits. The media and adventitia are unchanged.

Anatomic Diagnoses: Primary. Generalized arteriosclerosis involving particularly the coronary arteries; cardiac hypertrophy and dilatation; chronic passive congestion of the viscera; fibrosis of the myocardium; infected infarct, thrombus, and rupture of the left ventricular wall; hemopericardium.

CASE III. Clinical History. A white female, aged 66 years, with a known history of diabetes mellitus was admitted to the New Haven Hospital on February 24, 1926, with a complaint of precordial pain of two weeks duration. Four days before admission the previously dull pain suddenly became severe enough to cause the patient to faint and hospitalization was advised. On admission the pulse was 100 per minute, irregular and of poor volume; the respirations were 20 per minute and the blood pressure, systolic 132, diastolic 82. There was no dyspnea or cyanosis. The cardiac boundaries were not made out. A systolic murmur was heard in the apical region. The important laboratory findings were a leucocytosis of 24,500 per c. mm. with 85 per cent polymorphonuclear leucocytes. The electrocardiogram showed a regular rhythm with an average rate of 110 per minute and an inversion of a T wave in lead 1. On the day after admission a house officer found the patient's general condition improved. Twenty minutes later the patient died suddenly. The clinical diagnosis was diabetes mellitus and arteriosclerosis with special involvement of the coronary arteries; occlusion of the coronary arteries; infarction and rupture of the heart with hemopericardium.

Necropsy Report: The pericardial sac is enlarged and distended with about 200 cc. of fluid blood. The visceral pericardium is coated with a layer of recently clotted blood which varies in depth from 1 to 5 mm. The clot is lightly adherent to an area 5 cm. in diameter over the middle of the anterior surface of the heart. This part of the myocardium is softer than the surrounding muscle and is coated over with a fibrin deposit. There is a small irregular break in the pericardial tissue, measuring 2 to 3 mm. in any diameter, which extends into the softened infarcted area. The rupture is covered and filled with a lightly adherent blood clot. Before the heart is opened the coronary arteries are injected with a barium chlorid-gelatin mixture, and stereoscopic X-ray pictures are made of the injected heart. The injection material leaks out through the rupture in the anterior descending branch of the left coronary artery. The injec-

terial sympathectomy; resection of sciatic nerve, left); (b) coronary arteries with occlusion of descending branches, right and left; healed infarct of left ventricle; aneurysmal dilatation of infarcted area of left ventricle and rupture of its wall; hemopericardium; hypertrophy and dilatation of the left side of the heart; congestion of the viscera.

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tion of the right coronary artery shows no occlusion. The apex of the left ventricle shows very few injected vessels. The infarcted area is soft, opaque, brown-yellow, and is infiltrated with fluid and clotted blood.

Microscopic Examination: Just above the infarct the anterior descending branch of the left coronary artery is completely occluded by a recent thrombus and a thickened intimal and medial coat. Collections of mononuclear cells form dense collars about the vasa vasorum of the adventitia. The sections through the infarct show necrotic cardiac muscle fibers with vacuoles, loss of striation and nuclei, a diffuse and dense infiltration of polymorphonuclear leucocytes, acute inflammation of the vessel walls, a recent infected mural thrombus and an extravasation of red blood cells splitting up the cardiac fibers and extending out through the wall. A recently formed thrombus partly occludes the ruptured point. Sections elsewhere in the myocardium show an acute diffuse inflammatory process as well as areas of fibrosis and hypertrophy of the fibers.

Anatomic Diagnoses: Primary. Generalized arteriosclerosis involving especially the coronary arteries; fibrosis of the myocardium; thrombo-arteritis of the anterior descending branch of the left coronary artery; mural cardiac thrombus; infected infarct of the myocardium with hemorrhage and rupture; hemopericardium.

DISCUSSION

Krumbhaar and Crowell's review of 632 cases reported in the literature up to 1925 shows that 72 per cent fell in the age group of 50 years upward and about 4 per cent in the 30 to 40 year period. The greater number of cases were associated with preceding coronary artery lesions with a resulting infarction and rupture of the infarcted area. Invariably the site of rupture was in the left ventricle and was associated with changes in the anterior descending branch of the left coronary artery. Krumbhaar collected seven cases in 16,000 necropsies at the Philadelphia General Hospital and quoted seven in 13,000 at Munich, and nine in 8,000 at Leipzig. De la Chappelle reported twenty such cases among 15,059 necropsies from the Bellevue Hospital (New York City) and Medical Examiner's records. The three cases reported here are the total from 1,330 autopsies. Among these there have been 150 cases in which diseases of the

coronary arteries and the myocardium were the primary causes of death. In all but three of these cases the anterior descending branch of the left coronary artery was especially involved. Aneurysms of the left ventricle are rarely found. Kahn³ and Elliott⁴ present a review of the cases up to 1922 and their description of the typical aneurysm is similar to that of Case I.

The cases reported here present several interesting features. Cases I and III have associated diabetes mellitus and evidence of infection of the cardiovascular system. The etiology of the infection is not clear in either case. The anterior descending branch of the left coronary artery is completely occluded in each case, and in Case I the descending branch of the right coronary artery is also occluded. Evidence of old and recent infection of these vessels is presented. Case I is of further interest in that the patient was only 35 years old.

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DESCRIPTION OF PLATES

PLATE 64

FIG. 1. Occlusion of descending branches of the right and left coronary arteries; infarct of left ventricle; fibrosis of myocardium, rupture of aneurysmal dilatation of infarcted area; organizing and acute pericarditis.

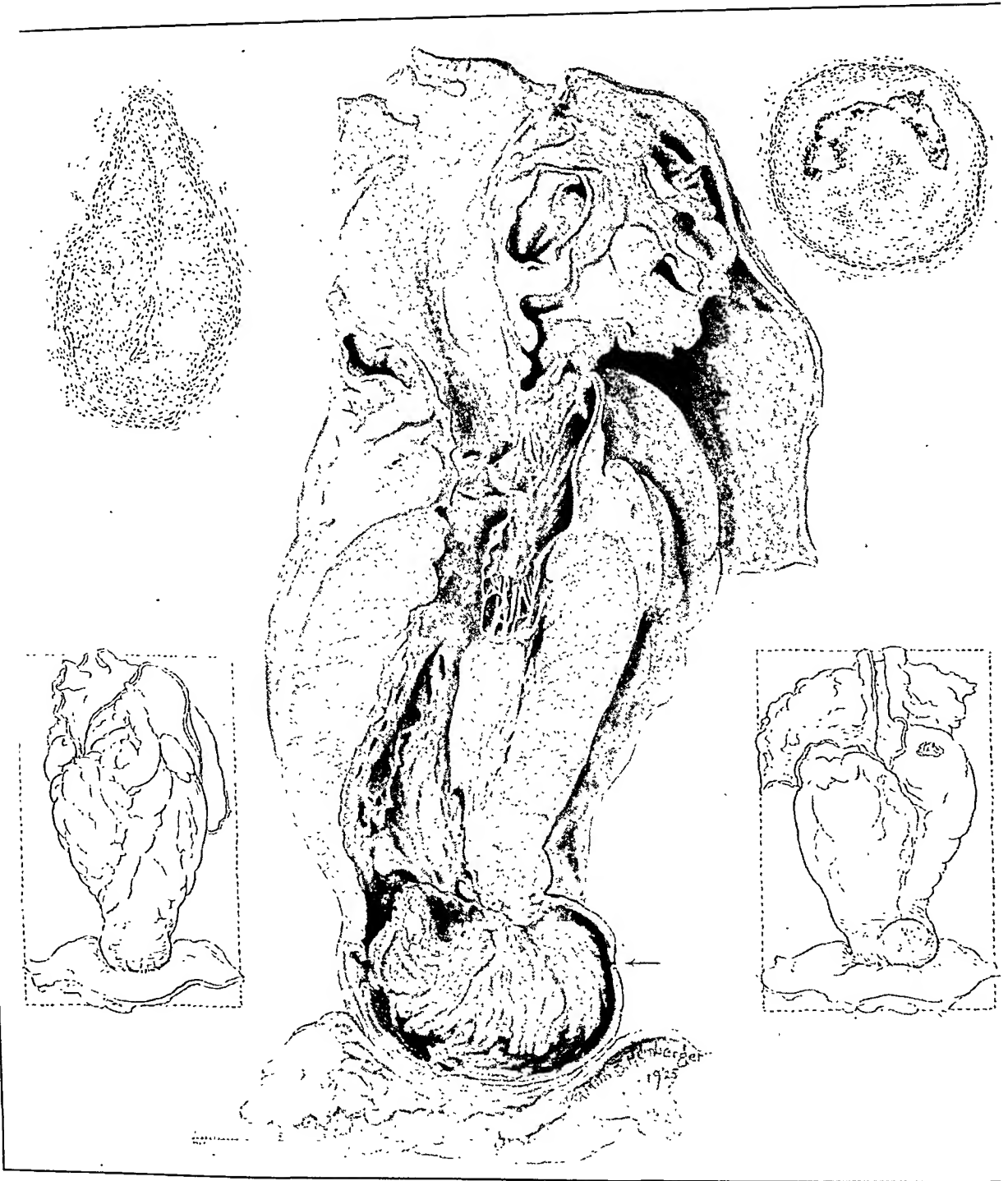


PLATE 65

FIG. 2. Infected infarct, thrombus and rupture of left ventricle wall (A).



PLATE 66

FIG. 3. Infected infarct of myocardium with hemorrhage and rupture.
nary thrombo-arteritis with occlusion.



3

THE EFFECT OF THE ORAL ADMINISTRATION OF POTASSIUM IODIDE AND THYROID SUBSTANCE ON THE MITOTIC PROLIFERATION AND STRUCTURE OF ACINI IN THE THYROID GLAND IN GUINEA PIGS *

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In previous papers Loeb has shown that, contrary to the current view, which is that administration of iodine prevents compensatory hypertrophy, this substance does not exert an inhibiting effect, and it may even increase the hypertrophy. Thus Loeb observed in a very extensive series of experiments that the average of hypertrophy was greater in the iodine-fed than in the control animals.^{1,2} This finding suggests that the primary effect of iodine on the thyroid gland is stimulating and that when a depressing effect is noticed, it is of a secondary nature; this conclusion applies to the normal thyroid. It appears that if during development of the organism there is a lack of iodine, this lack may also cause proliferation of the thyroid gland and a goitre develops. This proliferation can be prevented through administration of very small amounts of iodine. In certain cases the proliferation which has already begun can even be stopped through administration of iodine. On the other hand, in some instances, administration of iodine to goitrous individuals exerts a stimulating effect, causing proliferative processes and the appearance of toxic symptoms. Loeb concluded therefore that the thyroid tissue may respond to a deficit of iodine, as well as to a surplus of iodine, with increased activity and in particular with growth processes. Apparently the thyroid gland is adapted to a definite amount of iodine which enables it to function normally, and a diminution as well as an increase of iodine above this amount may disturb the normal cell equilibrium and act as a stimulus.²

In continuation of these investigations it seemed of interest to study the effect of administration of iodine on the intact thyroid

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† We are indebted to Miss F. L. Haven for assistance in these experiments.

gland, in which there is no lack of circulating hormone such as is caused by extirpation of a considerable portion of the gland tissue. Would administration of iodine under these conditions likewise act as a stimulus and call forth proliferative processes? In order to determine the effect in a definite and quantitative manner, we estimated the number of mitoses present at a certain time in the thyroid gland. We found that by this means we could establish in an exact degree the stimulating effect of iodine even in the normal gland.³ The depression in activity which takes place at a later period and under certain other conditions we attributed largely to pressure effects exerted on the epithelium lining the acini by the material contained in the lumina of the latter; but there may in addition be other factors involved. We have reason for assuming such pressure effects, because previously Loeb has observed in the thyroid of the guinea pig a breaking through of the walls of adjoining acini and a flattening of the lining-cells, evidently under the influence of pressure exerted by the colloid material. Thus the colloid of neighboring acini may form, in the end, one continuous mass; and it is very probable that many of the very large acini, which are found especially in the periphery of the thyroid gland, represent really the result of coalesced acini. It is probable that whole acini may thus disappear as a result of pressure and of consequent interference with the circulation. Similar observations can be made also in cases of human goitrous thyroids and pictures of this kind have been described previously by other observers.⁴ Furthermore, differences in the activity in the peripheral, middle and central zones of the thyroid of the guinea pig and the resulting differences in the pressures acting on the acinus cells in these areas are responsible for the development of these three zones of acini which Loeb described in the normal thyroid gland of the guinea pig. The factors mentioned, in particular the pressure exerted on the walls of the acini, explain the relative flatness of the epithelium in the peripheral acini.

In the experiments to be described we compared the number of mitoses found and the structural characteristics of the thyroid gland of normal guinea pigs serving as controls and of KI-fed animals; each of the latter received daily a dose of 0.05 gm. KI by mouth in the form of a pill.* In other experiments we fed a tablet containing

* In feeding the potassium iodide to guinea pigs care must be taken that the animals actually swallow the pill.

0.1 gr. desiccated thyroid gland (Armour & Co.) to the guinea pigs, a different set of animals serving as controls.

We carried out two series of experiments; the first series was made in April, May and early June, 1926; the second in December, 1926, and January and February, 1927, for control and potassium iodide-fed animals, while the experiments with feeding of thyroid gland were completed in April and in the early part of May, 1927. Thus we have to deal with three sets of guinea pigs in each series, namely: (a) controls, (b) guinea pigs fed with KI, and (c) guinea pigs fed with desiccated thyroid gland.

I. THE NUMBER OF MITOSES

We shall first discuss the results obtained in the second series, because here the method used for the determination of the number of mitoses was more satisfactory than in the first series.

SECOND SERIES: Five control guinea pigs, six guinea pigs each fed daily with 0.05 gm. KI and four guinea pigs fed with 0.1 gr. desiccated thyroid gland, were studied. The same kind of food was given to all. At the end of the experiment the thyroid glands were removed, immediately after death by chloroform, and fixed in Zenker's solution. Sections were cut serially.

In every tenth or eleventh section, the mitoses were counted and on this basis the approximate number of mitoses in the whole lobe estimated. In some animals an isthmus was present in the thyroid; it was usually small and not included in our counts.

(a) *Control Animals:* The numbers of mitoses found in one lobe of each of the control animals were as follows: 104, 80, 143, 63, 91. Thus an average of 96 mitoses per lobe or of 192 mitoses in the whole thyroid, excluding the isthmus, was obtained (see Table I). This represents the average number of cells which in our experiments were in mitotic division, at a certain time during the winter months, in the normal thyroid gland of guinea pigs, the weight of which varied approximately between 350 and 450 gm.

(b) *Potassium Iodide Animals:* Six guinea pigs which were fed with KI daily showed an average of 355 mitoses in one lobe or 710 mitoses in the whole thyroid, excluding the isthmus (see Table I). The lowest count in this series is more than twice as high as the average count in the controls and quite noticeably higher than the highest count in the control series.

(c) *Thyroid-Fed Animals*: In four guinea pigs, which were fed with 0.1 gr. thyroid gland daily for from 18 to 30 days, the counts of mitoses were as follows: 45, 0, 42, 40. The average count for one lobe was 32; for the whole thyroid, excluding the isthmus, it was 64 mitoses (see Table I).

FIRST SERIES: In the first and larger series, the number of mitoses was estimated in a different and less accurate manner than in

TABLE I
Mitotic Activity in Series II

KI-fed animals			Control animals		Thyroid-fed animals		
Guinea-pig no.	Duration of feeding in days	Mitoses*	Guinea-pig no.	Mitoses*	Guinea-pig no.	Duration of feeding in days	Mitoses*
10	16	868	79(a)	208	233	20	90
11	17	1008	79(b)	160	270	30	0
12	19	962	78	286	250	18	84
13	21	460	57	126	217	20	80
14	21	390	58	182			
15	23	572					
Averages of mitoses		710		192			64

* Mitoses, per whole gland, excluding isthmus.

the second series. In ten sections of the thyroid of each animal, the mitoses were counted in the central area, characterized by the larger size of the acini and the greater height of the epithelium. These central areas were, on the whole, somewhat smaller in the KI pieces, which were taken out for examination at later periods, than in the controls.

A larger number of sections were used for estimation of the number of mitoses in the thyroid glands of animals fed with thyroid substance than in the others to compensate for the smaller size of the areas in these thyroid glands. While, as stated, this method is less accurate than the method used in the later series, still the results agree fairly well in both and we may therefore consider the results here obtained as corroborative of those obtained in the second series.

(a) *Control Animals*: The results obtained are shown in Table II. The average number of mitoses counted in these 10 guinea pigs was 2.4. There were variations between 0 and 7 mitoses in the individual cases. The weight of the animals varied between 328 gm. and 870

TABLE II
Mitotic Activity in Series I

Control animals		
Guinea-pig no.	Weight in grams	Mitoses
134	695	5
218	660	7
104	580	1
345	515	1
458	620	1
332(a)	870	1
331	665	0
269	650	1
335	445	3
265	328	4
Average number of mitoses		2.4

gm., and the average weight was approximately 550 gm. The thyroid glands of these guinea pigs were removed for examination during the months of April and May.

(b) *Animals Fed with KI*: The average number of mitoses in all the animals in this series which were fed with KI is 6.3. The weight in the KI group varies approximately between 400 and 700 gm. However, there should be omitted from this list Guinea pig 212, which had been fed only during a period of 5 days with KI; the iodine effects were apparently not yet noticeable at this early period. Furthermore, we should omit Guinea pigs 315, 263 and 22 whose thyroids were removed as late as 108 days after the beginning of the

administration of KI. At this time marked retrogressive changes had begun in the thyroid gland. Omitting these four guinea pigs, the average number of mitoses of the remaining 13 guinea pigs is 8.1 (see Table III).

TABLE III
Mitotic Activity in Series I

KI feeding			
Guinea-pig no.	Weight in grams	Duration of feeding in days	Mitoses
212		5	2
216	768-773.....	8	8
217	865-850.....	10	7
264	410-423.....	12	12
267	390-375.....	15	8
328	520-585.....	21	15
268	520-547.....	25	7
386	465-513.....	30	5
41	485-600.....	30	1
37	535-580.....	30	9
306	465-595.....	40	14
367	525-605.....	40	6
300	415-485.....	60	3
330	393-420.....	60	10
315	735 end weight	108	0
263	650 end weight	108	1
22	815 end weight	108	0
Average number of mitoses (omitting Guinea pigs 212, 315, 263, and 22)			8.1

(c) *Animals Fed with Thyroid Gland:* Seven guinea pigs were fed with thyroid substance. The results obtained are presented in Table IV.

The weight in this group varies approximately between 500 and 600 gm. If we omit Guinea pig 420 in which, after only five days of feeding, the full effect of the thyroid substance had evidently not

TABLE IV
Mitotic Activity in Series I

Thyroid feeding			
Guinea-pig no.	Weight in grams	Duration of feeding in days	Mitoses
420	565-502.....	5	6
474	618-595.....	8	3
484	538-615.....	10	1
246	510 end weight	30	2
247	35	0
156	525-543.....	43	0
133	630 end weight	48	1
Average number of mitoses (omitting Guinea pig 420)			1.16

yet become apparent, the average number of mitoses in this group is 1.16.

Although the absolute number of mitoses is less accurately determined in the first series, it is of interest to compare the proportion in the number of mitoses in the controls, KI- and thyroid-fed animals in the first and second series. For this purpose we consider the number of mitoses in the thyroid-fed series and refer the number of mitoses in the other series to this unit number. In the first series, the proportions are as follows: Thyroid-fed group = 1; Controls = 2.1; KI group = 7. In the second series the proportions are: Thyroid-fed group = 1; Controls = 3; KI group = 11.

While the proportions found in Series II are more accurate than those obtained in the first series on the whole, the results in

both series agree. The number of mitoses is about three and a half times as great in the KI group as in the control group, and two to three times as great in the controls as in the thyroid-fed group. We may then conclude that during the first three to four weeks, and possibly even somewhat longer, KI feeding increases definitely and considerably the mitotic activity of the thyroid gland, and that on the contrary feeding with thyroid gland diminishes the number of mitoses (see Figs. 1 and 2).

II. THE SIZE OF THE ACINI AND OF THE CELLS LINING THEM AND THE CHARACTER OF THE COLLOID

(a) *Control Guinea Pigs*: In comparing the acini, the character of the acinus cells and of the colloid in the three series we must take into consideration the fact that noticeable variations may occur even in the control animals. Within the same group of animals the acini and the acinus cells present considerable differences in size. In the average control thyroid gland the acinus cells were medium to low, as far as their height was concerned. The colloid was slightly retracted; there were many vacuoles in the peripheral parts of the colloid; it was of medium consistency. The variations ran from large and medium-sized acini with low epithelium and with harder colloid that was slightly retracted, to acini of variable size, irregular in contour with relatively high epithelium and with colloid that was adherent to the epithelium in various places and separated from the latter merely by vacuoles.

(b) *Guinea Pigs Fed with KI*: If we compare the structure of the thyroid glands of guinea pigs fed with KI with the condition found in control guinea pigs, we find, during a period of approximately the first four weeks following the beginning of the experiment, no very decided differences between these two groups of animals. Again considerable variations in individual cases are present; while in some animals the average height of the acini cells may perhaps be less than in the controls, in others it is equal in the two groups, and in some cases it is even greater in the KI group. In particular, the thyroid of Guinea pig 10 fed with KI for a period of 16 days showed not only a relatively high epithelium but in addition colloid which, on the whole, was very soft and partly liquefied. This gland resembled the thyroids found in some cases of compensatory hypertrophy.

The thyroid of Guinea pig 12, examined after 19 days of daily KI feeding, showed acini with epithelial cells which were slightly higher than medium-sized; some of the acini had prominences extending into the luminae and were irregular in outline. The colloid varied somewhat in different acini; it was fairly hard in some, softer and even partly dissolved in others. In a number of acini it could be shown that the solution was produced, at least in part, through the activity of cells which phagocytosed particles of the colloid. The thyroid of Guinea pig 15, after a period of KI feeding extending over 23 days, was similar, although on the whole, the colloid was here somewhat harder and slightly retracted. Figure 3 (Guinea pig 328) shows the structure of the gland after a period of 21 days during which the animal received a daily dose of KI. The epithelium is of medium height in the majority of the acini, but in a number of the larger acini it is somewhat flatter evidently owing to pressure exerted by the contents of the acini on the layer of epithelial cells. The colloid appears, on the whole, soft and in a number of acini it has been invaded by phagocytic cells which have almost destroyed it.

These examples may serve to show that, for the most part, there is no marked difference in the size and character of the epithelium and of the acini between the control and KI guinea pigs within the first four weeks following the beginning of the KI feeding.

If we now consider the later stages of iodine feeding, we may state in general that there is a tendency for the acinus cells to become low; at the same time the colloid may be solid and be present in the acini in a relatively large quantity, but in other cases solution processes have taken place in it. There is, in addition, a tendency for the acini to become larger. These characteristics are quite definite in the specimens examined 108 days after the beginning of the feeding with KI. Fig. 5 (Guinea pig 315, fed with KI during a period of 108 days) illustrates this stage very clearly. The acini here are large, the epithelium is very low and the walls are thin. What is left of the colloid is very markedly retracted, much more so than in the earlier stages or in the control specimens. This marked retraction is probably a result of the action of fluid on the colloid and indicates softening processes which have taken place in this substance in certain of the acini. There is very little doubt, however, that pressure has been exerted on the walls of the acini by the colloid, or by the substances produced from the colloid through solution processes, and that this

pressure is largely responsible for the greater size of the acini and for the flatness of the epithelium at this period. In addition, we may assume that such an increase in pressure will lead to an interference with the circulation in the spaces between the acini. As a result of the increased intra-acinar pressure, which exists in the later periods of KI feeding, we find here and there that the walls of adjoining acini are flattened and broken through. Thus smaller acini may be united forming larger ones and, as Loeb has stated previously, the very large acini have been produced in many cases as the result of this secondary union of adjoining acini. The colloid of such acini may at first be united by a bridge passing through the openings in their walls, but it may finally form one connected mass. A transition to this condition is found in Fig. 4 (Guinea pig 37), where as early as 30 days after beginning the KI feeding some of the typical effects of the later periods of iodine on the thyroid gland are becoming noticeable. The acini are large, the colloid is correspondingly great in quantity, but still rather solid. The acinus cells, while on the whole fairly low, are distinctly higher than at later stages. The colloid here does not show the marked retraction seen in Fig. 5; in various places it still adheres to the wall of the acinus.

(c) *Effect of Thyroid Feeding on the Structure of the Thyroid Gland:* In the first week of thyroid feeding no definite differences were seen between the structure of normal glands and the glands of the thyroid-fed animals. From the tenth day on, there was on the whole, perhaps, a tendency on the part of the acinus cells to become somewhat flatter and for the colloid to be rather solid; the acini were, on the average, probably somewhat smaller than in the controls. However, these differences as a rule were not very pronounced and not present in all cases. The latest term at which the gland of a thyroid-fed guinea pig was examined by us was 48 days.

We shall cite as an example, the findings in Guinea pig 247, examined after 35 days of thyroid feeding. The acinus cells are here very low; the colloid is hard, almost entirely detached from the wall and rather markedly retracted. In some places the walls separating two adjoining acini have become very thin and they have the appearance of being ready to break through. Similar pictures of the rupture of walls of neighboring acini occur in thyroid glands in other instances and even in the thyroids of control animals, also wherever there is pressure exerted on the tissue separating two acini; this

process is therefore associated with flatness of acinus cells in the area in which such changes are taking place. The majority of the acini represented in this example are still relatively large, and in this respect the picture does not perhaps represent the average findings in the glands of thyroid-fed guinea pigs.

III. ON CERTAIN SECONDARY CHANGES IN THE THYROID GLAND

In a number of cases we found small accumulations of lymphocytes in the stroma of the thyroid gland. Furthermore, not infrequently, we observed phagocytic cells in the colloid of acini; these take up small particles of colloid into their cell bodies and here destroy it; as a result of this process the colloid may assume a honey-combed appearance. Colloid was also seen, in certain cases, in the interstitial connective tissue that separates the acini. The latter condition seems to depend on the destruction of the walls of some acini, perhaps as the result of pressure, and the subsequent escape of colloid into the interstices of the stroma. However, in interpreting such pictures, we must be aware of the possibility that colloid may be artificially squeezed out from the acini into the connective tissue spaces and we have to distinguish between these two occurrences.

If we now compare the three groups of guinea pigs as to occurrence and frequency of these changes, we find that the latter condition may be seen in all of them; the phagocytic activity, however, seems to be more pronounced in the KI-fed animals than in the controls.

DISCUSSION

Our experiments have thus shown that in the early stages of administration of iodine to normal guinea pigs, the number of mitoses is quite noticeably increased in the acinus cells of the thyroid gland and we may therefore conclude that iodine exerts primarily a stimulating effect on this gland. While we believe that the number of glands which we have examined is sufficiently large and that the results are sufficiently concordant to justify a general conclusion concerning this formative stimulation exerted by iodine in the case of the guinea pig, still we do not consider our observations will permit us to draw definite conclusions, as yet, as to the intensity of the

increase in cell proliferation, which we find here as the result of iodine feeding, or as to the average number of mitoses found in normal thyroids of guinea pigs. Further investigations, which are being carried out in our laboratory at the present time, may be expected to determine these conditions more definitely.

Of special interest in our investigations is the difference which we found between the action on the thyroid gland of iodine feeding and of the feeding with thyroid gland. Our observations in this respect correspond to the differences established in the influence exerted by these two substances in compensatory hypertrophy of this gland where feeding with thyroid (and to a lesser extent also with anterior pituitary gland) depresses, whereas feeding with KI stimulates, the proliferative processes in the acini. We may explain the difference between the action of these two substances in the following way: Feeding with thyroid gland introduces an excess of the hormone into the circulation and produces the effects observed in the thyroid gland of the animal. Such effects are the opposite of those which follow operative removal of a considerable portion of the thyroid gland tissue; the latter procedure leads to a diminution in the circulating hormone and stimulates growth processes in the remaining part of the thyroid gland; the former procedure depresses the activity of the gland, the function of which is now no longer needed and which might even become injurious. On the other hand, the administration of iodine is not identical with the introduction of ready-made hormone; in the former instance a substance has been introduced which is able to initiate the production of hormone in the gland cells, and it is apparently this stimulating process, set in motion by iodine, which also induces the gland to proliferate; this phase of action is necessarily lacking if we introduce the hormone as such. In a subsequent phase, when possibly an excess of hormone has been produced as a result of this stimulation of the thyroid gland, there may perhaps be added to the primary stimulation exerted by iodine, a secondary inhibiting effect due to the presence of an excess of hormone which may thus, secondarily, cause a depression in the activity of the gland.

However, whether this secondary inhibiting effect of hormone action comes into play at a certain stage and thus causes a depressive action we must consider problematical at the present time. On the other hand, there are definite indications that pressure is exerted

on the walls of the acini which produces the flattening of the epithelial cells, the enlargement of the acini, and in many cases the perforation of the wall of adjoining acini. And we may attribute to this factor, at least in part, the decrease in activity found in later stages of KI administration. This pressure is due to the action of the unchanged or partially liquefied colloid which is not removed from the acini of the gland in the same active manner in case of KI feeding, as it is in the condition found in compensatory hypertrophy where the removal of thyroid gland tissue, in some way, leads to a mobilization of the colloid and where thus neither the colloid as such nor the substance into which the colloid is secondarily transformed is able to the same extent to exert pressure on the inner lining of the acini.

The results formerly obtained by one of us concerning the effect of iodine on compensatory hypertrophy of the thyroid gland and his conclusions as to the primarily stimulating effect of iodine on this organ, as well as our present results regarding the effect of iodine on the normal gland, are at variance with the widely accepted view that iodine in general prevents proliferative changes in the thyroid gland and produces a quiescent condition of this organ. According to Marine and his collaborator,⁵ after once a goitrous proliferation and metabolic hyperactivity have started in the thyroid gland, administration of iodine leads to the production of an increased amount of colloid in the acini and thus ultimately a colloid goitre is produced, which under these conditions represents the ultimate resting stage of the abnormal gland.

SUMMARY

1. Oral administration of iodine to guinea pigs markedly increases the mitotic activity in the thyroid gland during the first three to four weeks.
2. This first phase of increased activity is followed by a second phase of depression, which is accompanied by definite structural alterations in the thyroid gland. We find indications that this second phase is at least partly due to pressure exerted by the contents on the walls of the acini.
3. In contradistinction to the effect of potassium iodide, administration of thyroid gland substance is not followed by a phase of

stimulation, but in this case, within the first two weeks, a period of depression sets in which may be accompanied by certain structural changes in the gland. As the result of thyroid feeding the mitotic activity is diminished as compared with that found in the controls.

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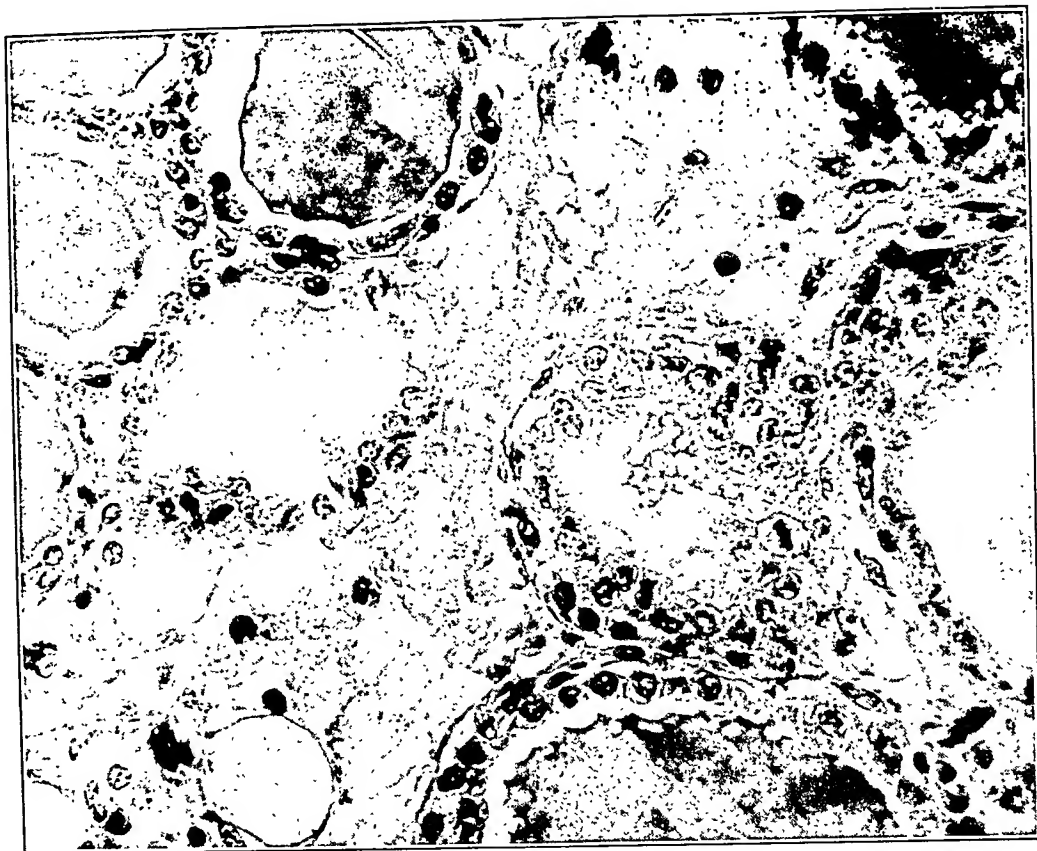
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DESCRIPTION OF PLATES

PLATE 67

FIG. 1. Guinea pig 11. 17 days KI feeding. Central part of gland, showing two mitotic figures, tall epithelium, softening of colloid.

FIG. 2. Guinea pig 11. 17 days KI feeding. Periphery of gland, showing two mitotic figures, one in center slightly out of focus. The epithelium is fairly high.



1



2

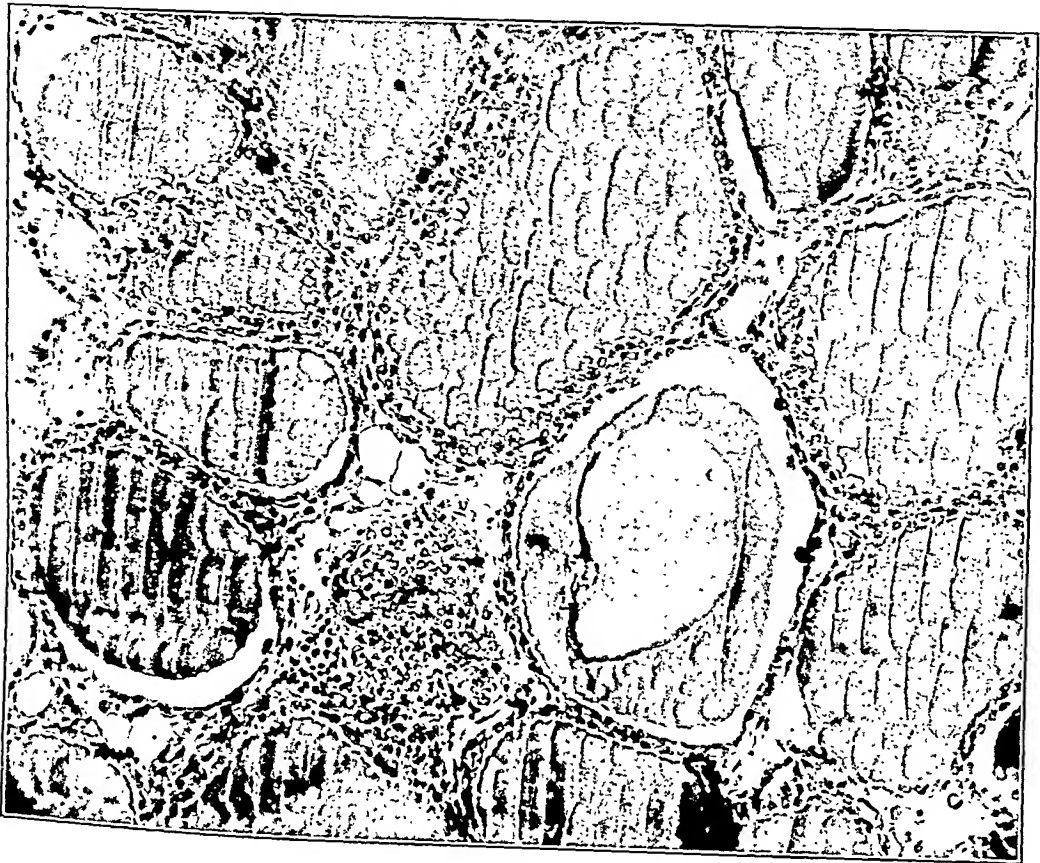
PLATE 68

FIG. 3. Guinea pig 328. 21 days KI feeding.

FIG. 4. Guinea pig 37. 30 days KI feeding. Beginning change to low epithelium, hard colloid.



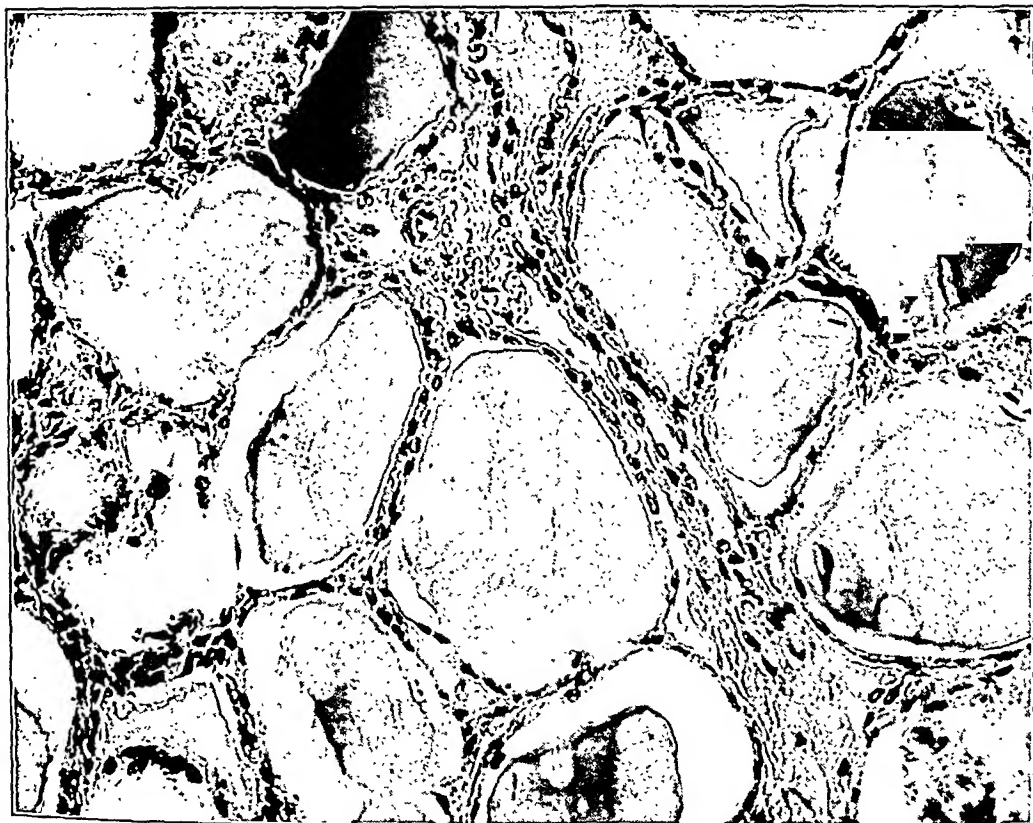
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4

PLATE 69

FIG. 5. Guinea pig 315. 108 days KI feeding. Flat epithelium, soft colloid.
In the place reproduced in this picture the acini were smaller than elsewhere in this gland.



5

OBSERVATIONS ON INCUBATED NORMAL BLOODS *

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The past five years have witnessed an increasing interest in the cells of the blood. The modern methods of treating the anemias and the conception that there may be a cell in the blood stream which reacts specifically to certain infections, such as tuberculosis, have stimulated a number of workers to investigations on the subject. The cell which has received particular attention is the so-called monocyte or large mononuclear cell of Ehrlich. This is probably because there is no unanimous agreement as to the classification, origin and physiologic activities of this cell. One method of attack on this problem has been the use of cultures of blood and of tissues in the hope that by observing the development and activity of the cells a proper classification could be arranged.

In recently published observations on cultures of leukemic blood ¹ we reported the development of two types of large cells. The question of the appearance of similar cells in cultures of normal blood was considered a matter of sufficient interest to warrant a series of experiments. The object of this work was to determine if the monocyte of the blood developed into an actively phagocytic large cell resembling the tissue phagocyte.

To make the discussion clear it is necessary to define the terms which are used in this paper.

MONOCYTE

This cell is the one called the large mononuclear or transitional cell by Ehrlich; monocyte by Nageli and Sabin, Cunningham and Doan; and endothelial leucocyte by Mallory and his school. Stained by Wright's method this cell shows an oval or indented nucleus somewhat eccentrically placed, which contains less chromatin and that more scattered than the chromatin in the nucleus of a lymphocyte. There is a considerable amount of cytoplasm which has a

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peculiar fine, faint, uniform neutrophilic granulation. The supravital spread method of studying the cells of the blood stained by neutral red and Janus green which was originated by Pappenheim² and brought into general use by Cunningham, Sabin and Doan³ has aided the study of these cells. The nucleus is mottled when observed in this manner and does not contain a nucleolus. The centrosomes located near the center of the cell are usually surrounded by mitochondria and granules which stain with neutral red. This grouping of red stained granules is called a rosette by Sabin and her co-workers. They state that this type of cell never phagocytoses material in the central region where the rosette is found but always at the periphery. They consider the phagocytic activity of this cell to be definitely limited as compared to that of others such as the clasmatocyte. The monocyte is actively motile though less so than the polymorphonuclear leucocyte. It has thin pseudopods and more cytoplasm in proportion to nuclear material than the lymphocyte.

LYMPHOCYTE

This term includes all the mononuclear cells of the blood except the monocytes. Stained by Wright's stain the nucleus is round or sometimes indented. There is a large amount of dense chromatin. The cytoplasm may be very variable in amount as compared to the nucleus. The color of the cytoplasm is a clear, light blue. A few azure granules may be present and occasionally a perinuclear clear zone is observed.

CLASMATOCYTE

This cell is the one called clasmatocyte by Ranvier, adventitial cell by Marchand, pyrrol cell by Goldman, polyblast by Maximow, macrophage by Metchnikoff, and histiocyte by Aschoff and Kiyono. It is a highly phagocytic, actively migratory cell found scattered throughout the connective tissue of the body. Wright's stain brings out a round or indented nucleus with considerable chromatin and a large amount of evenly stained blue cytoplasm. When observed by the supravital technic these cells show the cytoplasm packed with material of irregular size and distribution which stains with neutral red. The nucleus is round, oval or indented, often eccentrically placed and usually contains a nucleolus. Sabin states that this type

of cell contains no mitochondria. Lewis,⁴ however, found when films were stained only with Janus green all these cells contained mitochondria but when neutral red and Janus green were combined the heavy mass of red-staining material obscured them. This cell is actively motile and phagocytic. The tendency of the cytoplasm to form pseudopods is very marked.

CULTURE DATA

The first cultures of human blood are those described by Awrorow and Timofejewskij⁵ in 1914. They cultivated the blood of patients with myelogenous leukemia. They found that the myeloblasts gave rise to giant cells, clasmatoocytes, multipolar cells and cells similar to Maximow's polyblasts. In similar experiments on normal blood, no such cells developed. Therefore, they concluded that the presence of myeloblasts was necessary for the development of the large types of cells.

Timofejewskij and Benewolenskaja⁶ in 1927 reported cultures of the blood of a myelogenous leukemia patient who had eighty-four per cent myeloblasts in the blood. They felt that the myeloblasts gave rise to polyblasts, giant cells, clasmatoocytes, fibroblasts, granulocytes and probably lymphocytes. In this paper they mention cultures of normal rabbit blood. In these, they described polyblasts, giant cells and clasmatoocytes as arising from the monocytes and lymphocytes.

The first successful cultivation of cells of normal blood was that of Carrel and Ebeling⁷ in 1922. These workers coagulated the buffy layer of centrifuged blood by the addition of a few drops of embryonic juice. The resulting clot was handled like a tissue culture and the cells examined from time to time. They state that the polymorphonuclears and lymphocytes disappeared after the first week of incubation. Large ameboid cells developed which when stained by neutral red showed variable-sized red granules almost filling the cytoplasm. This cell was considered to be a clasmatoocyte. They mention the appearance of a cell of irregular shape, tending to be elongated, which contained very much finer granules staining with neutral red than the clasmatoocyte type. They considered this cell to be a fibroblast because groups would occasionally join to form a syncytium. These cells were found to have the same resistance as

fibroblasts⁸ to certain toxic agents, such as arsenic. No fibroglia or collagen fibrils were demonstrated.

In a study of the blood and subcutaneous tissues of the chicken these authors⁹ concluded that the monocyte of the blood and the clasmatocyte of the tissues were identical cells. Their variation in function and morphology depends upon environmental conditions, not on any difference in origin. They felt that one type could be changed into the other by altering the conditions under which growth took place.

Maximow has claimed since 1902 that the polyblasts or mononuclear cells which appear in inflammation arise in part from local cells, the resting wandering cells or histiocytes of the tissue, and in part from lymphocytes and monocytes which have migrated from the blood vessels. In 1924¹⁰ he described cultivation experiments using animal tissue. He observed the appearance of large, ameboid phagocytic cells and felt that he could trace their development from tissue histiocytes, blood monocytes and lymphocytes. In later experiments described in 1927 Maximow¹¹ cultured the leucocytic coat obtained from guinea-pig blood. Lymphocytes were seen to change in shape and size. Active ameboid movements with the development of pseudopods appeared and fat droplets were present in the cytoplasm. The monocytes were also seen to hypertrophy. He states that by the second or third day monocytes and lymphocytes indiscriminately had transformed themselves into large phagocytic polyblasts.

Fischer¹² in 1925 followed the technic of Carrel and cultivated a clotted buffy coat of centrifuged blood. Muscle, which had been kept in the ice box until the fibroblasts were no longer viable, was added to the culture. Pure cultures of cells were obtained, similar to those described by Carrel and Ebeling. Many granules of neutral red were seen in the cytoplasm. As the outline of these cells was irregular and elongated they were considered to be fibroblasts.

In 1924 and 1925 M. R. and W. H. Lewis,^{13, 14, 15, 16} together and separately, carried out extensive observations on the blood of a large number of species of animals. Their method was to allow a drop of blood to clot on a coverslip which was inverted over a hollow ground slide and sealed with petrolatum. The cultures were incubated at 37.5° C or kept at room temperature when they were from cold-blooded animals. To examine such specimens the plasma drop was

washed off with Locke's solution to which neutral red or Janus green had been added. Such preparations had to be discarded after study.

These workers attempted to follow the changes occurring in the various types of cells seen in the living blood cultures in order to determine just which cells were transformed into macrophages, epithelioid cells and giant cells. As controls, fresh cultures were examined shortly after their preparation in order to identify the various types of white cells with and without the aid of neutral red and Janus green, and to determine whether or not macrophages, epithelioid cells and giant cells were ever present in the normal blood. Such cells were never found in the first few hours. Though no special effort was made to determine how long the various types of cells lived and no sub-cultures were made the cells sometimes remained alive in the primary cultures three to four weeks. Very little change was seen in the granulocytes cultivated by this method. They developed a few neutral red-staining granules and occasionally phagocyted red cells. The lymphocytes showed an absolute increase in the number of neutral red-staining granules. Although any direct transformation of lymphocytes into mononuclears was not seen these authors state that the many intermediate stages between the two forms suggested that possibly such a transformation occurred. The monocytes were seen to become modified into macrophages, epithelioid cells and various intermediate forms. These workers describe mononuclears which showed active phagocytosis of red blood cells. Other mononuclears showed little phagocytosis but accumulated a large number of small neutral red-staining granules about the centrosome. These structures gradually became larger until they almost filled the cell. The mononuclears increased in size and often fused to form giant cells. They frequently were irregular in shape and showed long pseudopods.

These workers concluded from their long series of experiments that mononuclears, macrophages and epithelioid cells are merely different phases of the same cell type.

The work of two other investigators should be considered in this connection. Kindwall¹⁷ presented evidence that monocytes were not present in lymph from the thoracic duct. Bloom¹⁸ made clot cultures of thoracic duct lymph and observed the cells which developed by both supravital spreads and fixed, sectioned preparations. He saw the appearance of large cells containing a well marked

rosette when stained with neutral red. There were numerous transition forms between these cells and lymphocytes. The large cells showed many granules staining with neutral red and some lipid droplets in the cytoplasm. They were actively phagocytic and a certain number of long spindle-shaped cells appeared. When fixed and stained they showed a basophilic cytoplasm and a larger, more vesicular nucleus than the lymphocytes.

METHODS

The methods described below were usual in our work. The use of plasma clot cultures makes repeated observations on the same preparation impossible. By employing a fluid culture medium we have been able to follow the same cultures for long periods.

Blood was drawn under aseptic precautions from the median basilic vein of various workers in the laboratory. Rabbits were bled from the heart. About 1 cc. of blood was placed before clotting in small test tubes. In some cases 2 cc. of blood were added to 1 cc. of a 1:200 dilution of heparin in Locke's solution giving a final heparin dilution of 1:600. Occasionally 1 cc. of the blood-heparin mixture was diluted with 9 cc. of Locke's solution. Some of the rabbit blood was mixed with one per cent sodium citrate in equal proportions: 2 drops of this mixture were added to 1 cc. of rabbit serum. Where a culture of clotted blood was desired the tube was ringed with a sterile loop as soon as a moderately firm clot had formed. The tubes were sealed with paraffin and incubated at 37.5° C. In some cases control tubes were kept in the ice box. Particular care was used to keep the tubes away from the light. Observations were made from time to time by removing a drop of sediment from the bottom of the tube with a platinum loop. This method permitted repeated observations to be made of the same culture.

Each sample was observed in a supravital spread and air-dried smears were usually made at the same time. Most of the supravital slides were prepared with neutral red only as the toxicity of Janus green prohibited its routine use. Observations with Janus green were made whenever there was any question as to the presence or absence of mitochondria. The dried smears were stained by Wright's blood stain and by the method of Sato and Sekiya¹⁹ for the oxidase reaction. A few of the clot preparations were fixed in Zenker's fluid,

embedded in paraffin, sectioned, and stained with eosin-methylene blue. Occasionally preparations which had been stained supravitaly were pulled apart and stained by Wright's method. Supravital observations on preparations which had been in the warm box more than thirty minutes were considered invalid.

The presence or absence of hemolysis was found to be good evidence as to the presence of living cells. If the tube showed no hemolysis it was fairly safe to prophesy that no living cells would be found.

RABBIT'S BLOOD

Granulocytes: These cells could be followed with little difficulty until the sixth day and sometimes longer. From the second day on they became progressively less motile and exhibited neutral red granules of irregular size and a few large unstained refractile granules in the cytoplasm. Usually from the fourth to the sixth day it was very difficult to identify them positively. By the sixth day nearly all were rounded up and unstained. The dead forms could frequently be seen in the Wright's stained preparations at the end of two weeks incubation. The oxidase reaction remained positive long after the death of the cells. The eosinophiles persisted almost unchanged for weeks.

Lymphocytes: The small lymphocytes showed very little change during a long period of incubation. They could usually be followed to the second week and sometimes longer. The tendency to develop an increased number of granules staining with neutral red as described by Lewis was not particularly marked. Transition forms between small lymphocytes and monocytes were never seen. The morphology remained constant as observed in the fixed preparations.

Monocytes: During the first three days the neutral red-staining rosette of these cells became more definite and larger as the granules increased in size and number. After twenty-four hours, uniform, round unstained granules began to appear in the cytoplasm. These granules increased in number as the time of incubation went on. They tended to be arranged at the periphery of the cell. They reacted for neutral fat with Nile blue sulfate. By the fourth day these cells were of good size and phagocytosis was quite active but variable. From that time on the cells increased in size until a maximum diameter of about twenty-five to forty microns was attained.

A rosette of good sized, red-staining granules near the nucleus persisted and often the cytoplasm was completely filled with unstained refractile granules. Stained by Wright's method these cells showed a round to oval nucleus, fairly vesicular in type. The cytoplasm was blue in color and contained a large number of unstained vacuoles.

X Cells: At about the fourth day a type of cell appeared which was somewhat different from any seen in the blood up to that time. Observed by the supravital technique the cytoplasm contained a large number of fine red granules suggesting the hypertrophied rosette mentioned by other workers. Refractile granules were present occasionally but were fewer in number and finer than those seen in the cells described as monocytes. Phagocytosis was very active; the cytoplasm often contained great masses of red-staining material and cells. Multinucleated forms were frequently seen. The outline of these cells was quite irregular. Many stellate and elongated forms were present and the tendency to form pseudopods was well marked. Motility usually persisted until the end of the second week when most of the cells became quite sluggish and tended to be round. Stained by Wright's method the nuclei appeared round and contained a fair amount of chromatin. There was a considerable amount of even blue cytoplasm. Some basophilic granulation was present occasionally and often masses of red-staining material. The oxidase reaction was both positive and negative.

As far as could be determined there was no consistent difference according to the conditions under which the cells were kept. There was a suggestion that the cells in the tubes diluted with Locke's solution changed more slowly than those in the clotted tubes. The same types of cells were found irrespective of the method used.

Bloods kept in the ice box showed none of the changes exhibited by those in the incubated specimens. The cells remained alive and active until about the fourth week.

HUMAN BLOODS

Granulocytes: Observed by the supravital method the neutrophils contained an increasing number of red-staining masses and a few unstained refractile granules in their cytoplasm after the first day. They gradually became less motile and more distorted in shape.

By the fifth day they were so changed that recognition was impossible and nearly all were dead. Only a rare one was seen after this time. Stained by Wright's method they could be recognized for two weeks but were extremely misshapen. The oxidase reaction persisted long after the death of the cells.

The eosinophiles showed almost no change and could be followed sometimes as long as two weeks.

Lymphocytes: The small lymphocytes persisted in a living state as observed by the supravital spread method until about the tenth day. There was a slight tendency for the red granules to increase in size and intensity of staining as the period of incubation was prolonged. After the tenth day the outline of the cells could be seen but they were considered to be dead as indicated by the lack of motility and of red-staining granules. In the Wright's stained preparations they could be followed unchanged for two weeks. There was practically no variation in morphology. The oxidase reaction was consistently negative.

Monocytes: The changes which took place in this type of cell were followed from the first two hours of incubation to the death of the cell. In some cases daily observations were made. There was usually little question as to the identification of the monocytes and their derivatives. By the end of twenty-four hours incubation many had developed a variable number of round, unstained refractile granules. The focus of neutral red particles making up the rosette became somewhat larger and the granules more numerous as time went on, but the diffuse, finely granular, hypertrophied rosette was not seen in this cell. The focus of red granules usually persisted until the death of the cell. The cells became gradually larger by phagocytizing material which stained with neutral red and by the development of more refractile granules. These stained like neutral fat with Nile blue sulfate. By the sixth to the eighth day the characteristic picture was the presence of a variable number of cells measuring from ten to forty microns in diameter. The nucleus was indented and contained no nucleolus. More than one nucleus was rarely observed. A focus of rather coarse red granules was usually present. The remainder of the cytoplasm was filled with fairly uniform, clear, unstained granules among which globules of red-staining material were scattered. Motility was sluggish at all times but was observed as late as the tenth day of incubation. The cell outline

tended to remain quite rounded; true elongated and stellate-shaped cells of this type were not observed.

Stained by Wright's method these cells had a very characteristic appearance. They stood out sharply against the background of granular *débris* on account of the unstained vacuoles. Every transition could be observed between the twenty-four hour old monocyte with a few unstained granules and the large cell seen after six or more days of incubation with the rosette persisting and a cytoplasm honeycombed with unstained areas. No transitions were seen between the monocyte and any other type of cell seen in the cultures. When the oxidase reaction was applied to these cells it was usually positive.

In discussing this type of cell later in the paper we will speak of it as the Y cell.

X cells: From the third day of incubation on, a cell appeared which was unlike any seen in the blood before incubation. When first seen it was actively motile and possessed an oval nucleus often with a nucleolus. The number of nuclei varied from one to four. The cytoplasm was much more irregular in shape than that of the monocytes. Stellate and tremendously elongated forms were observed. The cytoplasm contained a large number of fine, uniform, somewhat brown-red granules which were concentrated near the nucleus in a rather dense mass suggesting a rosette. The granules of this structure were consistently finer and more evenly distributed than were those of the monocyte. Phagocytosis was very active and sometimes the whole shape of the cell was distorted by great masses of phagocytosed material. Refractile unstained granules were rarely present and if seen were very fine, of irregular size and few in number. As the period of incubation increased this type of cell became increasingly larger, in many cases attaining a diameter of sixty to seventy microns. Forms containing from two to five nuclei were observed and coalescence of single cells of this type to form giant cells could often be watched.

In the dried smears stained by Wright's method these cells could be seen very clearly. There was a round to oval nucleus with fairly dense chromatin and a variable amount of clear blue cytoplasm. As incubation continued, these cells took up a large amount of phagocytosed material in their cytoplasm. Red and white blood cells and irregular masses of hemoglobin could be seen. They often dis-

played a few basophilic granules near the nucleus. Multinucleated giant cells were common. As time went on these cells took up more and more foreign material until at the end of the second week of incubation little was seen but a great mass of red-staining material and a nucleus. Transition forms between this type of cell and the monocytes were not seen. This cell is often negative to the oxidase reaction but not infrequently one containing an oxidase-reacting granule was seen.

DISCUSSION

From the foregoing data we may conclude that large phagocytic cells develop in incubated rabbit and human blood. Two types of these cells were seen. From observations made at different periods of incubation it is possible to make some deductions as to the source of such large cells. No change was seen in the small lymphocytes or the granulocytes which would lead one to believe that they ever increased in size and became phagocytic. The only remaining possibilities are the blood monocyte or some other type of cell existing normally in the blood stream. Such cells have been described by Ferrata as the hemohistioblast, by Maximow as the hemocytoblast and by Sabin as the clasmatoocyte. The blood monocytes could be followed accurately from day to day. They retained their small, distinct rosettes although the granules increased somewhat in size and number. Refractile granules which showed as unstained vacuoles in the Wright's stained preparations appeared at the end of twenty-four hours and increased in size and number thereafter. Phagocytosis was not particularly active and multinucleated cells of this type were not often seen. The oxidase reaction was nearly always positive.

The second type of large phagocytic cell appeared about the third or fourth day of incubation. When first seen it was very actively motile with long pseudopods. The size was that of a blood monocyte or somewhat larger. Phagocytosis was very active. Observed in supravital spreads the cytoplasm contained a large number of very fine, diffuse red granules, sometimes tending to group in one area, suggesting the hypertrophied rosette described by some workers, and sometimes quite uniformly distributed throughout. Multinucleated forms were often seen. Refractile granules were fewer and smaller than those seen in the monocyte derivatives. When stained by

Wright's method these cells displayed one or more round to oval nuclei with a moderate amount of chromatin. There was a large amount of uniform blue cytoplasm containing a few unstained vacuoles. Most of these cells were negative to the oxidase reaction although those showing a positive reaction were not uncommon. As the period of incubation increased the difference between these two types of cells became less marked. As observed after a week or two most of the cells seen contained a large amount of phagocytosed material and a variable number of refractile granules. So much red-staining material was present in the cytoplasm that the structure of the cells was obscured.

Three general schools exist with different theories as to the source of the large phagocytic cells which develop in incubated blood. Maximow since 1902 has claimed that a primitive cell exists in the tissues and blood which is morphologically like a lymphocyte and which may become an actively phagocytic large cell, a lymphocyte or a granulocyte. Bloom has demonstrated the development of large phagocytic cells in cultures of lymph from the thoracic duct. Since Kindwall and others have shown that lymphocytes are the only cells present in the contents of the thoracic duct it is difficult to conceive of any source for the large cells except cells of the lymphocyte type. Our experiments with cultures of human lymph nodes which will be described in a later paper tend to bear out this theory. Ferrata²⁰ has described a pluripotential cell which is sometimes present in the blood stream and has called it a hemohistioblast.

The Lewises after extensive observations on bloods of different species of animals concluded that the only source of large phagocytic cells was the blood monocyte. As their large cells phagocytosed actively and the engulfed material was irregularly distributed through the cytoplasm they concluded that it was the same as the phagocytic cell of the tissues described by Sabin and called a clasmatocyte. They felt that the monocyte and clasmatocyte were simply functional variations of the same cell.

Sabin and her co-workers explained the development of large phagocytic cells of the clasmatocyte type in incubated blood by stating that endothelial cells or their derivatives were normally present in the blood and gave rise to the large cells. They believed that the monocyte never attained such a large size or phagocytosed as actively as the clasmatocyte type cells.

Without discussing the origin of these cells which we will take up in a later paper we have concluded from our observations and from the literature that there are probably two sources for the large phagocytic cell, one, a primitive cell normally present though unrecognizable in the blood; two, the blood monocyte. We think that it is possible for a time to separate the two types of large cells developing from these two sources by certain differences in activity, in staining, in morphology and in phagocytosis. After a certain period of incubation the differences become less marked and many cells of an indeterminate type are seen.

From this premise we feel that the theories of all three schools may be correlated and made to agree with the existing facts which are so difficult to explain on any one theory above.

If the theories of the Lewises were correct, it would be very difficult to account for the oxidase-negative large cells, since ninety-five per cent of human monocytes are oxidase-positive. Should the theories of Sabin, *et al.*, be accepted, the large cells should be oxidase-negative, save for granules of phagocytosed material. Neither of these two schools could explain the results of Bloom. Timofejewskij and Benewolenskaja, following the teachings of Maximow, believed the large cells were derived from a primitive, pluripotential cell, the hemocytoblast. Our work would tend to confirm this view. The existence of such a cell would account for the varying oxidase reaction of the large cells and the results of Bloom.

CONCLUSIONS

1. The large phagocytic cells developing in incubated rabbit and human blood may be derived from two sources.
2. These large cells show certain differences in morphology, in staining and in activity for a time which make it possible to group them in two classes.
3. One group arises from a primitive pluripotential cell normally present though unrecognizable in the blood stream.
4. The second group develops from the blood monocyte.

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GENERALIZED RETICULAR CELL SARCOMA OF LYMPH NODES ASSOCIATED WITH LYMPHATIC LEUKEMIA *

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Leukemia, or even a leukemoid blood picture, is an unusual occurrence in the course of tumors. When the cells in the blood are morphologically identical with those of the tumor, a genetic relationship between the blood picture and the organ changes is generally assumed. If (less frequently) the leukemic cells are of entirely different structure from those of the tumor, a relationship is less firmly established. In every instance the interpretation is difficult, and the diagnosis frequently in doubt.

The case which forms the basis of this communication is one in which lesions thought to be those of an unusual tumor of the lymphatic apparatus, are associated with those of lymphatic leukemia.

REPORT OF CASE

Clinical History: W. H., Shipping clerk, age 46 years. Entered Bellevue Hospital June 14, 1926, complaining of swelling on the left side of neck, duration seven weeks.

Family History and Past Personal History: Irrelevant.

Present Illness: Seven weeks ago the patient noticed a swelling on the left side of the neck which increased gradually in size. It was not painful. The patient had occasional pains in the epigastrium and suprapubic regions, of short duration, which had no relation to meals, defecation or exertion. He had lost a great deal of weight in the last two months.

Physical Examination: (Positive findings only.) Adult white male, appears chronically ill. Marked emaciation. Eyes: Petechial hemorrhages in palpebral conjunctivae. Neck: Masses of nodes in left cervical region, anterior and posterior chains. The individual nodes appear to be about 2 cm. in diameter. There are smaller ones in both supraclavicular regions. The nodes are firm and

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apparently discrete. Abdomen: Distended. Spleen edge 10 cm. below costal margin, firm, surface feels nodular. Liver edge at level of umbilicus, hard, smooth. Lymph nodes: Cervical, axillary, epitrochlear, inguinal, all enlarged, firm, discrete, not tender.

Blood Examination: Red blood count, 3,700,000; hemoglobin 60 per cent; white blood count 98,400; polymorphonuclear neutrophils 7 per cent; eosinophiles 0.2 per cent; lymphocytes 90 per cent; monocytes 2 per cent; myelocytes 0.6 per cent; plasma cells 0.2 per cent. There are many degenerated white cells in the smear. There is slight anisocytosis and pallor of the red cells.

Clinical Diagnosis: Lymphatic leukemia.

Course in Hospital: Progressive, downward. Died July 6, 1926.

NECROPSY

B. H. No. 11643. Summary of positive findings. There is generalized lymphadenopathy. The abdominal nodes form a retroperitoneal mass which extends from the diaphragm to the pelvis and from spleen to right kidney. The mass is composed of nodes which are 0.2 to 8 cm. in diameter, mostly discrete. Nodes in the mesentery and groin, cervical and axillary regions are also enlarged. On section all are very soft. Some are white or gray, some mottled with red, a few definitely hemorrhagic in whole or in part. Some nodes have central areas of the same consistency, but of canary yellow color. None is necrotic.

Spleen: 23 by 15 by 10 cm. Capsule is smooth, surface coarsely nodular. On section, there are numerous white, gray or yellow nodules measuring up to 2 cm. in diameter, of the same consistency as the lymph nodes. Many of these nodules have a hemorrhagic periphery, some have canary yellow centers. There is recent infarction.

Liver: Enlarged. Weight 2660 gm. It is pushed to the right and rotated by the peritoneal mass so that the left border is near the midline. There are numerous white spots on section, most less than 0.1 cm. in diameter, but some measuring up to 1.5 cm.

The nodules in the liver and spleen have the same appearance and consistency as the lymph nodes.

Intestine: In the ileum is a small ulcer, about 0.5 cm. in diameter, involving only the mucous membrane. It appears to be directly over a lymphoid follicle in the submucosa.

Bone Marrow of Rib: Hyperplastic, grayish red.

Bones: Normal, except for a fibroma of the periosteum on dorsal vertebra, 6.5 cm. in diameter.

MICROSCOPIC EXAMINATION

Lymph Nodes: None of the lymph nodes examined is normal. Two types of histological lesions are observed:

(A) The general architecture of the node is preserved. The lymphoid tissue is markedly hyperplastic. The normal distinction between follicular and medullary areas is lost, the whole being over-run with lymphocytes, forming large areas separated by trabeculae. There are no germinal centers.

The cells are nearly all lymphocytes of the small variety. Only a few large lymphoid cells are seen. Mitoses are present, but not common.

The blood and lymph channels contain an abnormally large number of small lymphocytes. The surrounding fat tissue is infiltrated with the same cells. The endothelium and reticular tissue are normal.

(B) In the other type of lesion, there are numerous polymorphous "endothelioid" cells, sometimes closely packed, more often loosely arranged, which have the following characteristics:

The cells are several times as large as the lymphocytes. Their nuclei are large and vary in shape. Hypertrophied forms and giant cells with nuclei resembling those of megakaryocytes are present in very variable numbers.

The nuclei have definite membranes, several prominent nucleoli, and a fine chromatic network.

The cytoplasm is abundant, often with protoplasmic processes. It is moderately basophilic, particularly with the basic blue stains, less so with hematoxylin. It is finely granular, but no special leukocytic granules are present. The benzidine peroxidase reaction in these cells, as in the lymphocytes, is negative.

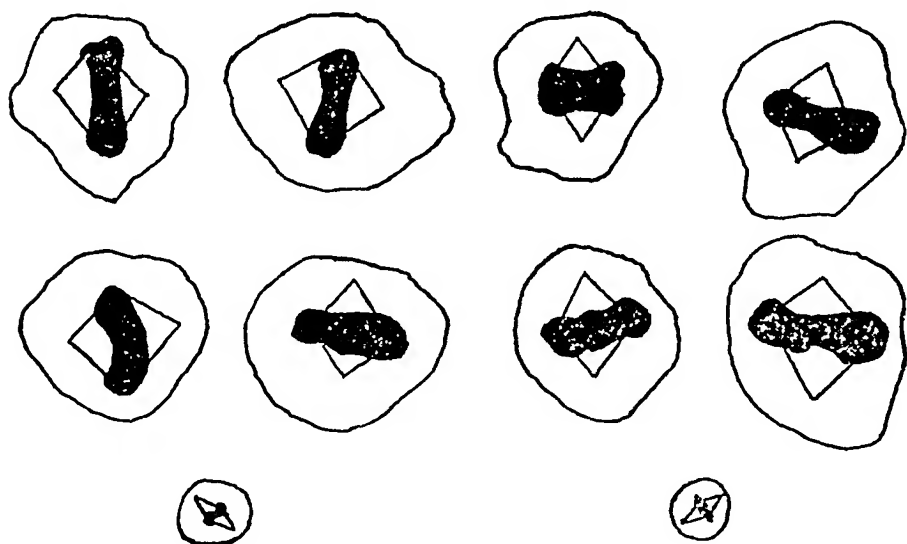
An interesting and occasional very conspicuous cytoplasmic constituent is the centrosome, which is distinctly stained, particularly by phosphotungstic acid hematoxylin (black), and by azure-eosin (red). This structure is single or double, sometimes multiple. From the centrosome are radiating lines (astral rays) which, under suitable resolution, appear granular.

The centrosome and astral rays are present not only in dividing cells, but also in many at rest. The centrosome, in cells with indented nuclei, is sometimes situated just within the nuclear indentation, or

in the cytoplasm on the same side of the nucleus. If the cell has cytoplasmic processes a centrosome may often be seen in each process if the angles are included in the plane of section. In dividing cells, a centrosome is situated in each mitotic angle.

The angle of mitosis is, on the average, 84° (Text-Fig. 1).

These cells are obviously of connective tissue origin, though the cells from which they arise can be determined with neither ease nor accuracy. They seem to have no genetic relationship with the



TEXT-FIG. 1. Mitoses in tumor cells and in lymphocytes, showing characteristic variations in the mitotic angles. The two small cells are lymphocytes, the others are from the tumor. Camera lucida sketch.

lymphocytes. No conversion of lymphocytes into the polymorphous cells, or vice-versa, could be found, although the two lesions are frequently found together. Neither is there any definite evidence of their origin from endothelium. Frequently they are seen just outside the lymph vessels, with intact endothelium between them and the lumen. Often, also, they are found in the situation where lymph sinuses ordinarily would be found.

I am inclined to regard these polymorphous, "endothelioid" cells as arising from the reticulum, particularly that part known as reticulo-endothelium, in the lymph nodes.

The two types of lesions are found in all the groups of nodes examined, almost always in the same nodes. Only occasionally is a section seen with only one lesion, *i.e.*, purely leukemic, or purely neoplastic. Sections through two adjacent nodes may show an

abrupt change from a leukemic to a neoplastic lesion, not an extension from one node to another.

Liver: The liver has the same two types of lesions that are found in the lymph nodes: leukemic and neoplastic.

The leukemic lesion consists of periportal lymphomata of different sizes, and smaller intralobular lymphocytic collections. These lesions are identical with those usually seen in lymphatic leukemia.

The other lesion consists of tumor nodules of the same cell type described in the lymph nodes, which form large areas completely destroying the liver tissue, and infiltrating the surrounding liver lobules. Sometimes these tumor cells are seen in the center of a leukemic collection, especially in the vicinity of a large tumor mass, but no transitions between the two cell types can be identified.

Spleen: The nodules observed in the gross specimen are composed of collections of the same type of cell described in the lymph nodes and liver as "tumor cells." These collections are mainly rather compact, completely destroying and replacing the splenic tissue. Smaller collections of the same type of cells are present in the splenic pulp.

The malpighian corpuscles are not observed, the remaining splenic tissue consisting of numerous small lymphocytes scattered without definite arrangement.

Intestine: The small ulcer is directly over a nodule in the submucosa which evidently was a lymphoid follicle. Only a small portion of the lymphoid tissue is present, the remainder being obliterated by tumor cells identical in appearance with those described above. The main mass of the tumor is confined to the follicle, but a few tumor cells can be seen infiltrating the mucosa. The remaining lymphoid tissue is too small in amount to determine whether or not it is the seat of leukemic change.

Kidney: There is hyaline degeneration of the tubular epithelium. In a few areas are poorly defined collections of small lymphocytes.

Tumor of Periosteum: This consists of closely packed, elongated fibroblasts and collagen fibers arranged in bundles which run in different directions. The cells have no resemblance to either the lymphocytes or to the cells of the generalized tumor. In a few places there are collections of small lymphocytes.

Bone Marrow: Unfortunately, examination of the marrow was confined to that of the ribs and vertebrae. In those situations, the marrow is very cellular, the predominating cell type being the small

lymphocyte. In a few areas, small clusters of myeloid cells remain. The picture is regarded as typical of the marrow in chronic lymphoid leukemia. Tumor cells were not observed.

My interpretation of this case is as follows:

The patient had chronic lymphatic leukemia for a much longer time than the history indicates. Subsequently there developed a rapidly growing, malignant tumor arising from reticular and reticulo-endothelial cells of the lymphoid tissues. The tumor developed in, encroached upon, and destroyed tissue which was previously the seat of leukemic changes.

EPICRISIS

On the Leukemic Lesion: It is known that blood pictures resembling leukemia may occur as a result of different conditions, among them tumors. It is also known that in cases with the gross and microscopic lesions of leukemia, the blood picture may give no indication of this condition. We must, therefore, regard the blood picture in leukemia (or in any other condition) as a *symptom* which may vary within wide limits and which does not in any sense constitute the disease itself. A diagnosis of leukemia based solely on the blood picture may, therefore, be questioned, but when combined with the typical features of leukemia in the various organs, it must be regarded as established.

In other words, the diagnosis of leukemia rests, in the final analysis, on tissue changes without which the disease cannot be said to exist, regardless of the blood findings. When these changes are present, leukemia may be diagnosed regardless of the apparent cause, for we are not able at present to establish the diagnosis of leukemia on an etiological basis.

Applying these statements to the present case, we may diagnose the presence of leukemia because of the typical histological changes in the organs together with a leukemic blood picture. Whether the leukemia is due to the tumor or is a disease *sui generis*, is another question.

On the Tumor: There can be little doubt that the lesion described in the foregoing account as a "tumor" should be so considered. The classification of the tumor, however, presents greater difficulties.

Although arising primarily in lymphoid tissues, the tumor is composed neither of lymphocytes nor their immediate precursors.

This is borne out by a study of the cells in both section and smear preparations, in neither of which do the tumor cells resemble any variety of normal blood corpuscles or their formative cells. For this reason, I think the term "lymphosarcoma" is not applicable.

The tumor is apparently derived from reticular and reticulo-endothelial cells. It has no connection with ordinary vascular endothelium, and is therefore not an "endothelioma." Certain properties normally possessed by reticulo-endothelium, however, are missing. For example, the cells do not produce reticulum fibers. In silver impregnation methods, an increase in the amount of reticulum is not demonstrated, nor is there a very intimate relation between cells and fibers. Phagocytosis is not observed, and abnormal cytoplasmic inclusions are not found in the tumor cells. This, however, does not disprove their origin from reticulo-endothelium, as it is known that tumor cells do not necessarily carry on the functions of the tissue from which they arise.

It would appear, therefore, that we are justified in regarding the lesion *in its final state* as a tumor probably arising from reticulum cells. Although it cannot be proved that the lesions in their incipency were of the same nature as those observed at necropsy, nevertheless to postulate a preëxisting lesion of another type (which has been transformed), is to read into the picture signs which are not there.

On the Relation of Leukemia to Tumor: The two lesions in this case have been referred to as "leukemia" and "tumor" for purposes of description. The fact that leukemia may itself be a tumor, does not enter into this discussion.

It is possible that the development of one of the lesions was dependent on the existence of the other. This question, however, involves a discussion of the etiology of both leukemia and tumors, and cannot be elaborated here. There is nothing in the foregoing account that precludes such dependence, and the fact that the tumor was encroaching on tissues formerly the site of leukemic changes, points in that direction.

The complete independence of the two lesions is conceivable, but not susceptible of direct proof. On one point, however, the histological evidence is conclusive: there is no evidence of the transformation of the cells of one lesion into those of the other. Lymphocytes and tumor cells, although intimately intermingled, are morphologic-

ally distinct. Cells in the blood stream are identical with those of the leukemic lesions, but strikingly different from the tumor cells.

This difference is also seen in a study of the "mitotic angles" of dividing cells. Ellermann¹ has observed that cells in mitosis have mitotic angles characteristic of the cell type. Petri² has confirmed this, and has used the mitotic angle to differentiate cell types in leukemia. Thus myeloblasts have angles of about 66° to 70° ; neutrophil myelocytes about 68° to 70° ; erythroblasts 21° to 22° ; lymphocytes about 45° . Measurements of the mitotic angles of cells in this case showed that in lymphocytes the angle is about 45° , but in the tumor cells, 84° . There is considerable variation among the different cells of the tumor, and individual angles were found to vary from 69° to 108° , but none was found to be as low as in the lymphocytes. The average angle observed in the tumor does not correspond to any reported in blood cells.

That this case is not a "leukemic transformation" of another condition is evident from a study of the sections, which indicates that the leukemia was probably the lesion first to develop. At the time of death, the tumor was much the more actively growing lesion. The wide distribution but relative quiescence of the leukemic lesion indicates long existence and slow development.

The evidence presented by the microscopic preparations thus enables one only to diagnose the *presence* of two lesions, without giving any definite indication that they are genetically related.

I am indebted to Dr. Francis Carter Wood for the photomicrographs illustrating Figs. 3 to 8, inclusive.

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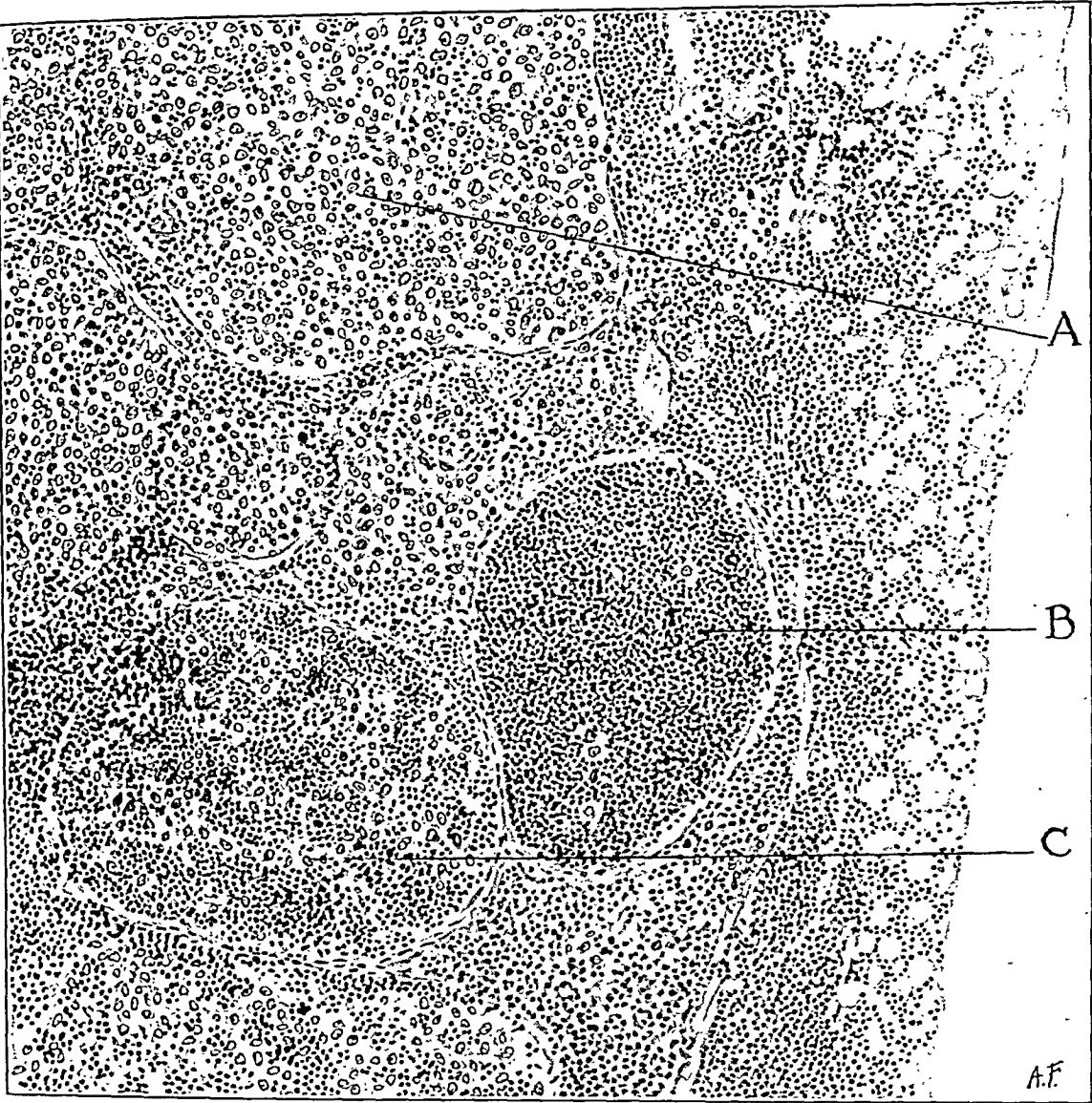
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DESCRIPTION OF PLATES

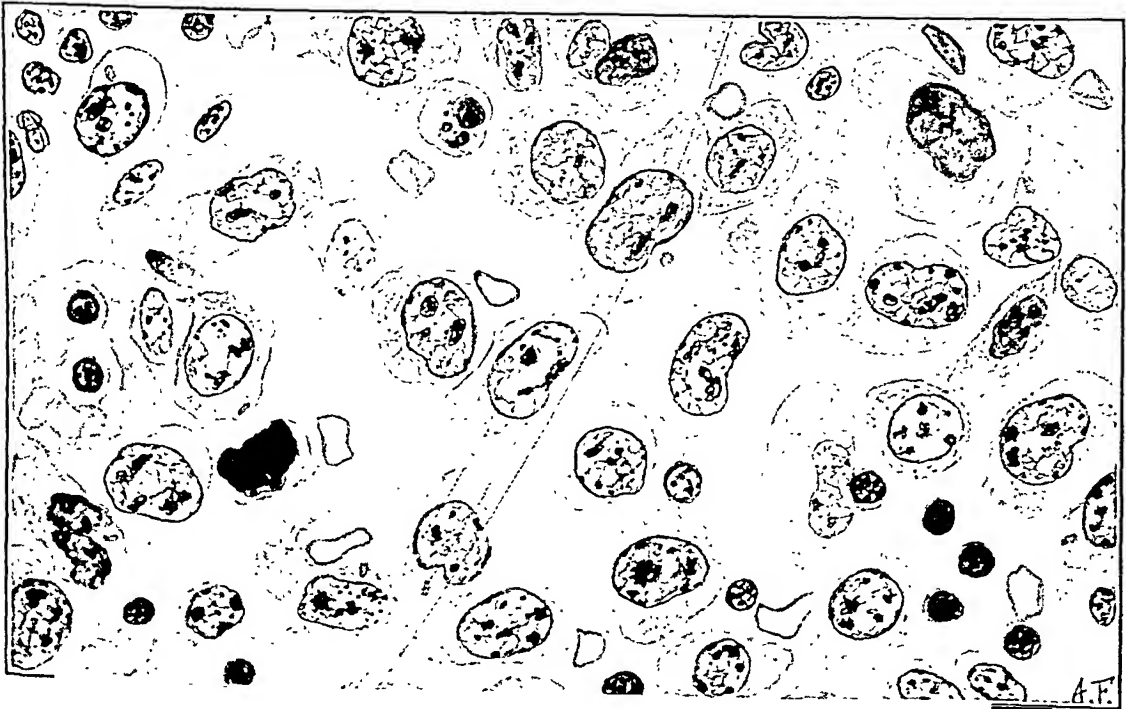
PLATE 70

FIG. 1. Lymph node. (A) area of almost complete replacement by tumor; (B) remains of leukemic lesion; (C) partial replacement of leukemia by tumor.

FIG. 2. Lymph node. Higher magnification of a tumor area.



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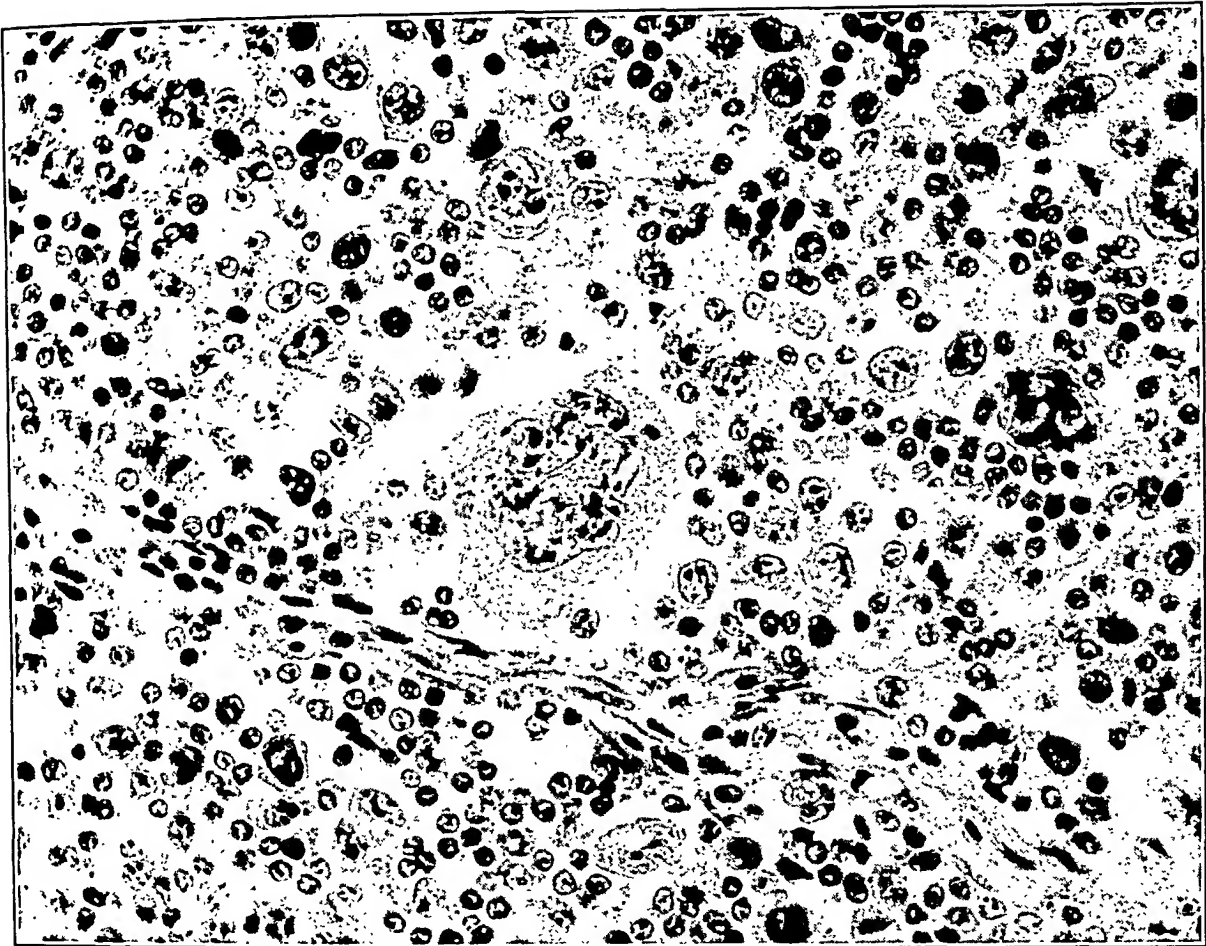
Richter

Reticular Cell Sarcoma and Lymphatic Leukemia

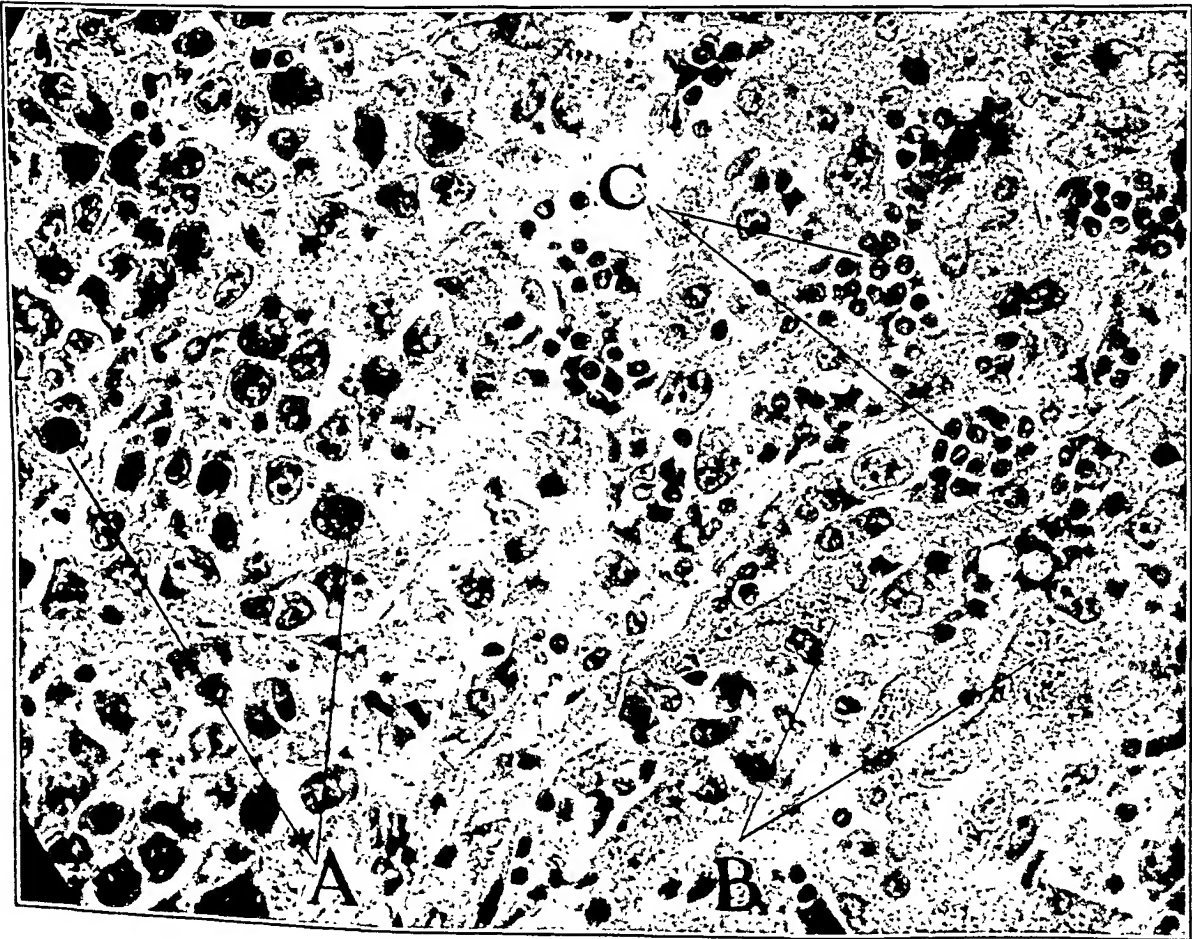
PLATE 71

FIG. 3. Lymph node. A tumor giant cell and other tumor cells intermingled with lymphocytes. $\times 500$

FIG. 4. Liver. Edge of a tumor nodule. (A) tumor cells; (B) liver cells; (C) collections of leukemic cells (lymphocytes) in the capillaries. $\times 500$



3



4

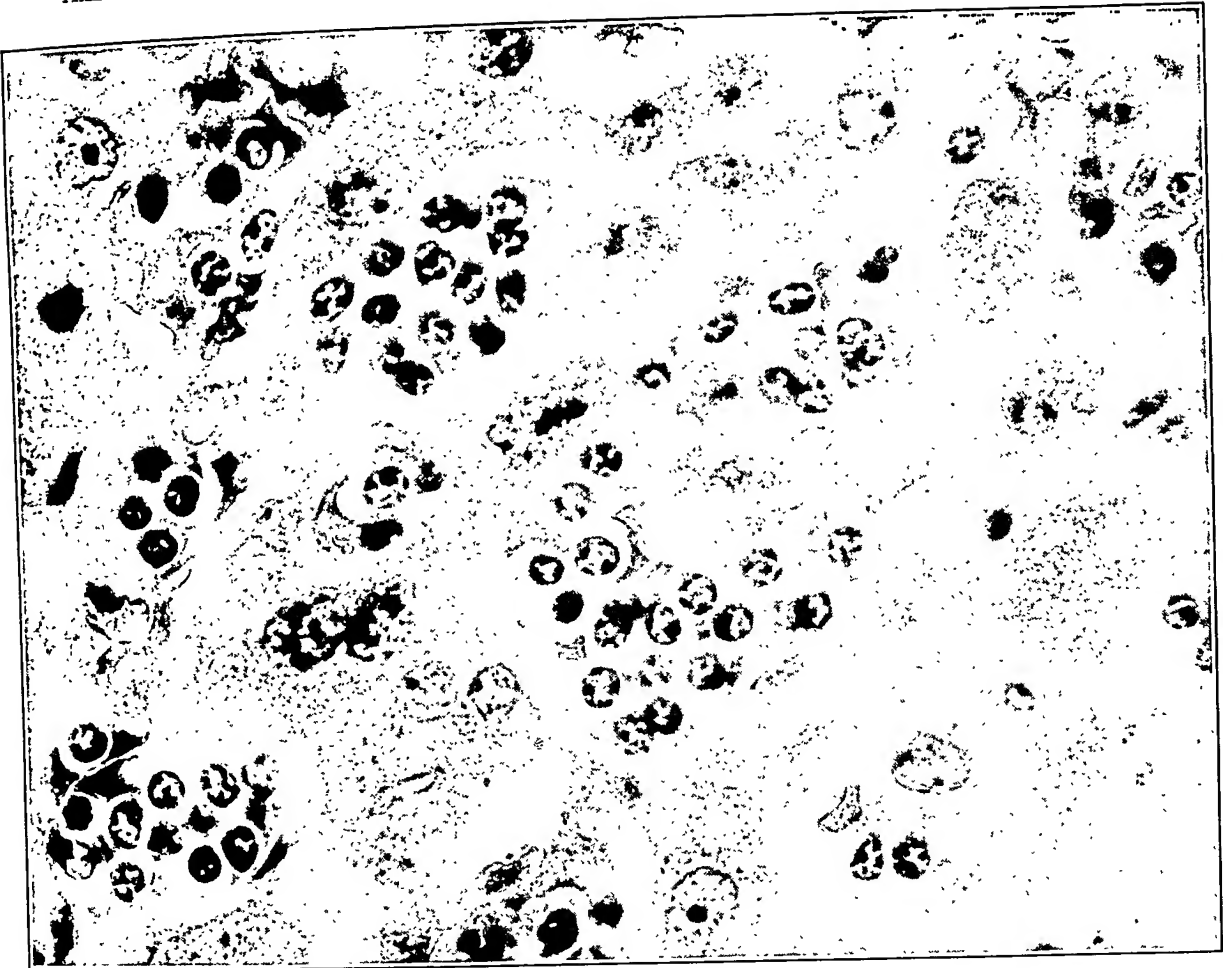
Richter

Reticular Cell Sarcoma and Lymphatic Leukemia

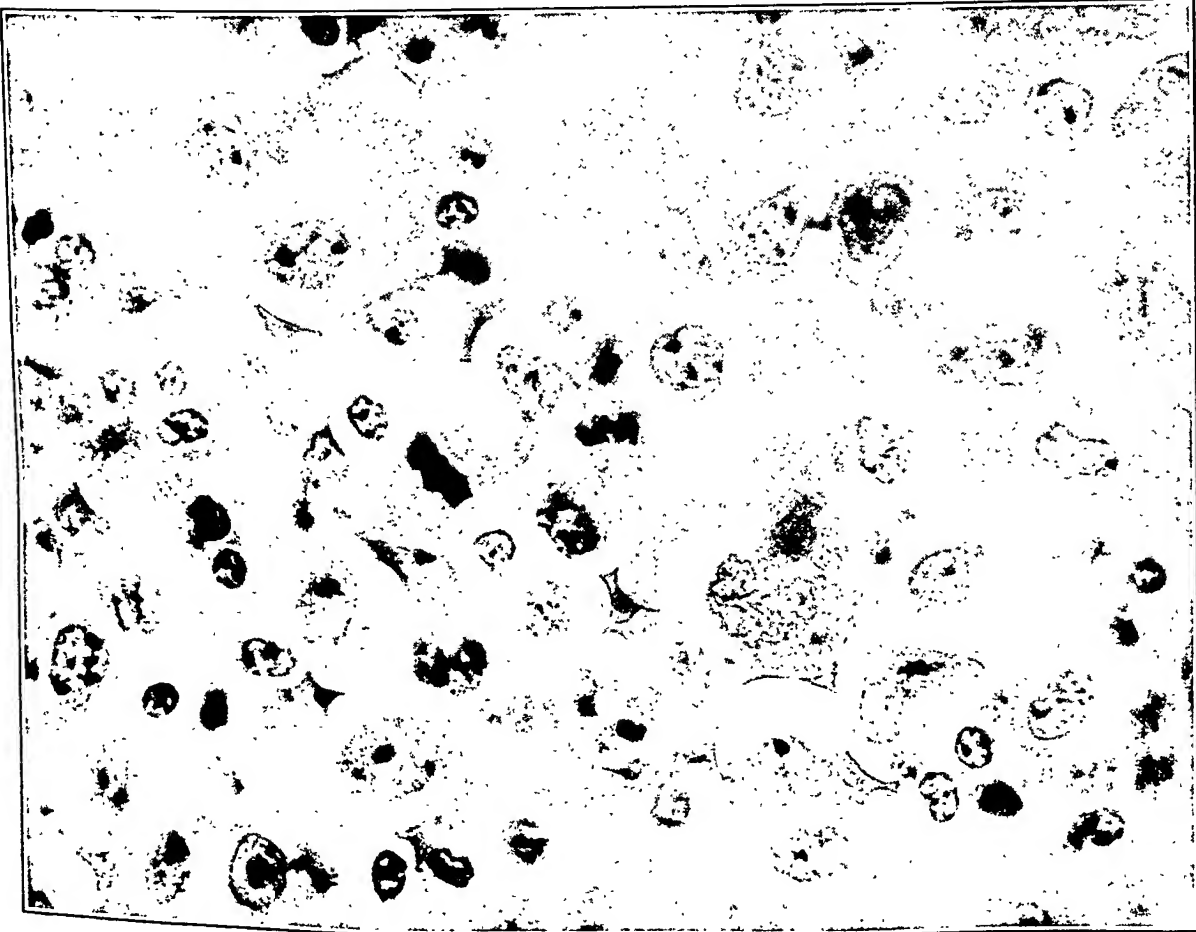
PLATE 72

FIG. 5. Liver. A higher magnification of the leukemic collections. $\times 1000$

FIG. 6. Liver. A portion of the tumor under the same magnification as Fig. 5.
Compare the size and structure of tumor cells and lymphocytes. $\times 1000$



5



6

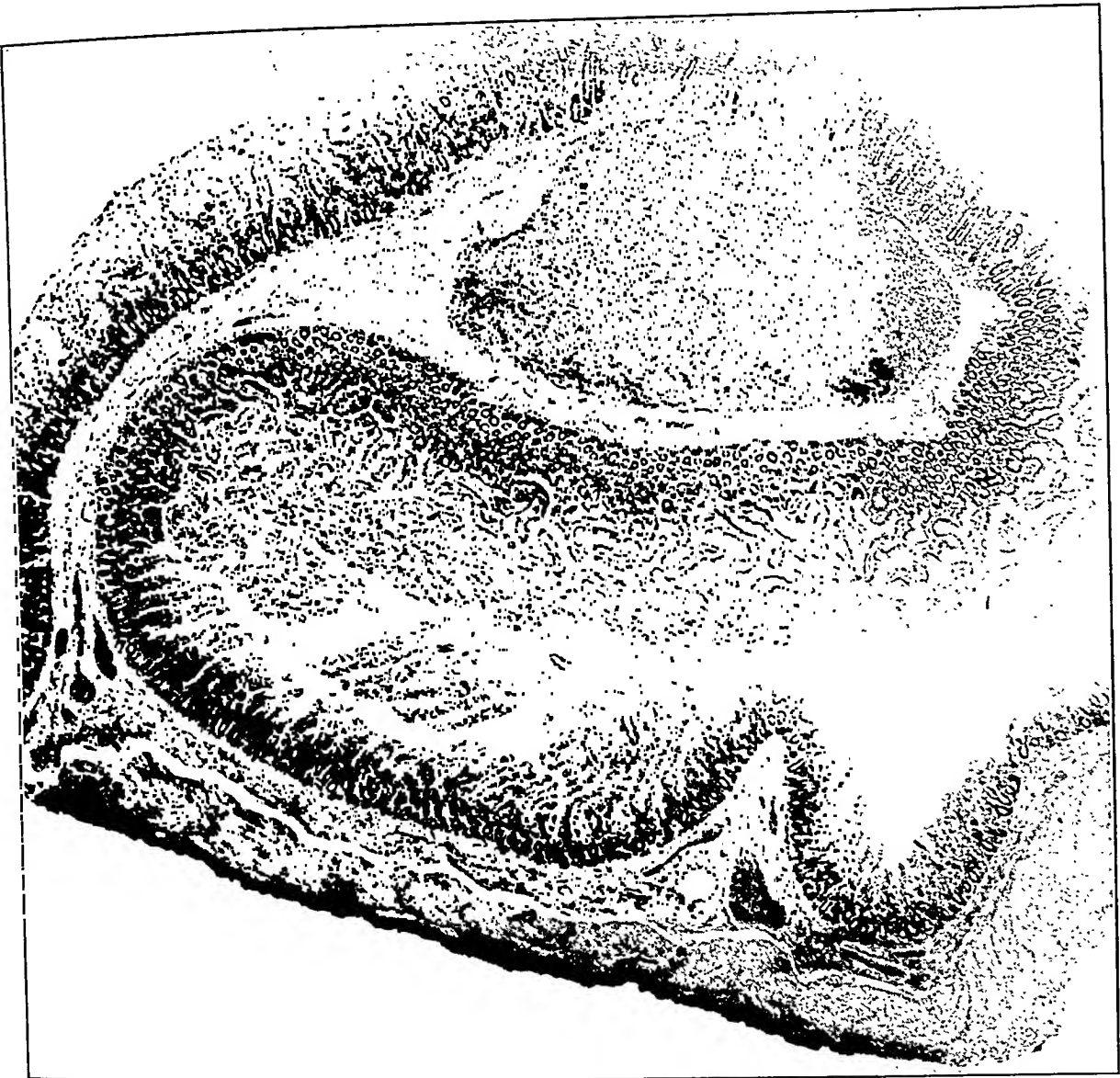
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Reticular Cell Sarcoma and Lymphatic Leukemia

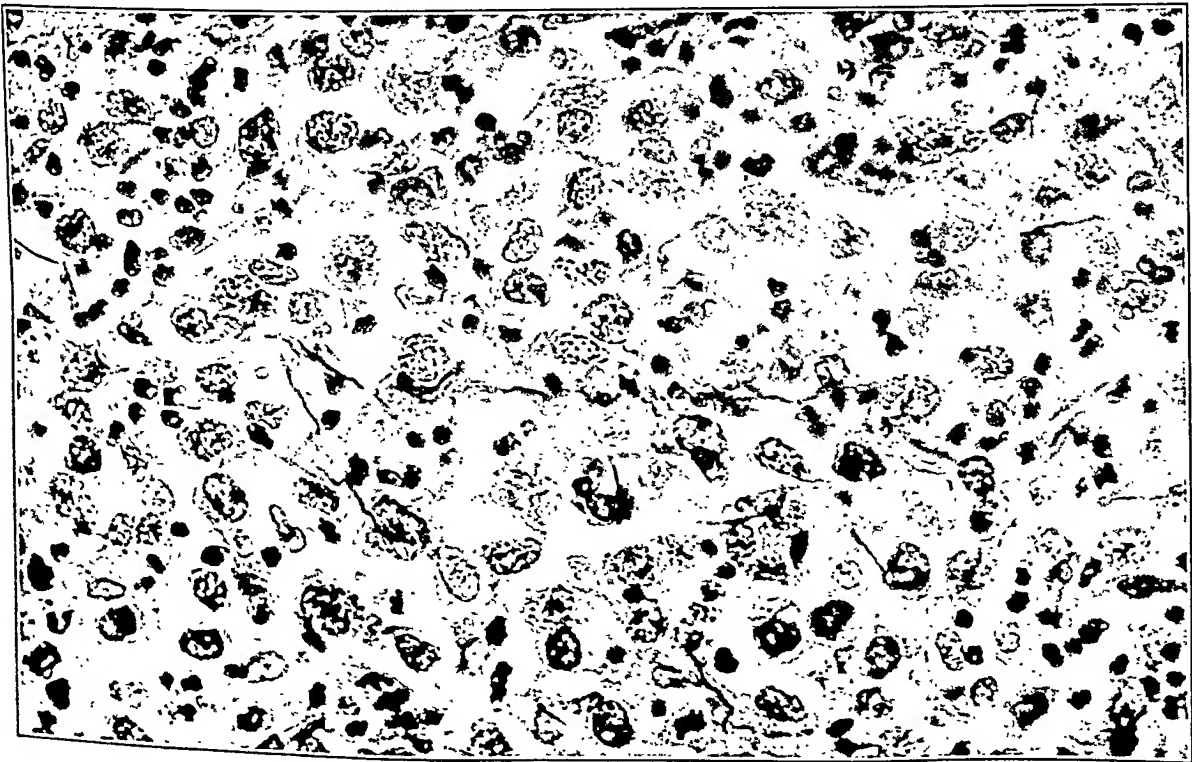
PLATE 73

FIG. 7. Intestine. Submucous nodule composed of tumor cells. $\times 20$.

FIG. 8. Lymph node. Stained for reticulum fibers.



7



8

Richter

Reticular Cell Sarcoma and Lymphatic Leukemia

STUDIES ON LIPOCHROMES *

IV. THE NATURE OF THE PIGMENTS IN CERTAIN ORGANS

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In previous papers the works of Willstätter and Stoll,¹ Van den Bergh and Snapper,² Palmer and his associates,³ and others, have been referred to, and the methods, in part, by which they have established the identity of the lipochrome pigments with the carotin and xanthopyll of plants have been mentioned. I have been able to demonstrate the presence of carotin by chemical means in the liver, spleen, adrenal glands, corpus luteum, skin and fat, using a method which seems to be satisfactory for its quantitative estimation, at least for purposes of comparison.⁴ But many tissues which are commonly said to contain lipochrome are not amenable to chemical examination because of their close association with other carotin-containing tissues, or because of the small amount present in the body. It was therefore necessary to resort to histologic methods to demonstrate the presence or absence of the pigment in such tissues. By the application of methods previously described,⁵ and by chemical examinations, I have been able to confirm the presence of lipochrome in the tissues just mentioned but could not demonstrate it satisfactorily in the heart, ganglion cells, seminal vesicles, or in any other tissues which are commonly said to contain this substance. The pigment present in these last organs, as is well known, is a yellow to brown granular substance which is frequently tinged with fat stains, and therefore has been called lipochrome in this country, and lipofuscin in Germany. These two names are used to designate the substance in most English and American literature, but they actually represent different pigments. Borst originated the term "Lipofuscin" (Hueck⁶) because he thought it was derived from some sort of lipoid. Sehrt⁷ named it "fat-binding wear-and-tear pigment" (*fettthaltige Abnutzungspigment*), and Lubarsch⁸ used this term for over twenty years. From time to time during this period the question has been taken up in Lubarsch's laboratory and varying results

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reported by Brahn and Schmidtmann,⁹ Salkowski,¹⁰ and Staemmler.¹¹ Salkowski, and Brahn and Schmidtmann devoted their time to chemical examinations, comparing the substance to melanin. They found that it agreed well so far as elementary analysis is concerned, with the latter pigment, both containing carbon, hydrogen, nitrogen and oxygen in about the same proportions. The pigment which they analyzed contained no iron. Staemmler, using a combined iron cyanide and silver nitrate method found that all these pigments (of heart muscle, seminal vesicle, even adrenal cortex) could be blackened and he concluded that they were melanin or forms of melanin. Block,¹² however, insists that melanin is formed only in ectodermal or mesodermal melanoblasts, the first found in the epidermis, retina, scattered cells in mucous membranes and the nervous system, the second (mesoderma) in the choroid of the eye, and rarely in the corium. Melanin is present also in phagocytic cells (melanophores) in the corium but is not produced by these cells. Masson¹³ agrees essentially with this, differing only in the name of the cells which form the pigment. Melanin is found only (Masson) in cells of the nervous system, and, while these are widely scattered, they can be differentiated from others by their morphologic and staining characteristics. Wells¹⁴ says that melanin is probably not found even in pathologic conditions in cells which normally do not produce this pigment.

There are therefore, as regards melanin, two schools of thought: one that melanins form a group of pigments, all closely related, some formed in cells, others as the result of degenerative processes in tissues (Lubarsch), and the other that melanin is a specific substance elaborated by specific cells which, in man, are mostly of ectodermal origin, and in lower animals are of mesodermal and ectodermal origin (Bloch, Masson, and others).

Hemofuscin is a term used by von Recklinghausen¹⁵ to name the yellow pigment found associated with hemosiderin in hemochromatosis. This is a yellow-brown pigment occurring in cells (endothelial or epithelial) or in the muscle and connective tissue cells of the liver, pancreas, intestinal wall and other organs in great abundance in this disease. Von Recklinghausen described brown atrophy of the heart and liver as localized hemochromatosis because of the presence of this pigment. Lubarsch and his school never recognized the name, but from the time of Sehr's work in 1904 until recently, they have

considered it a fat-containing "wear-and-tear" pigment and adopted Borst's term, lipofuscin, for it. Recently, as has been mentioned, Lubarsch and his co-workers have tried to prove it to be melanin. Hueck identified hemofuscin with lipofuscin in 1912 and considered it to come from a lipoid, probably fatty acid. It was not until Mallory became interested in hemochromatosis that the name hemofuscin was restored.

Mallory, Parker, and Nye¹⁶ have shown that this pigment is constantly associated with hemosiderin in hemochromatosis in a large number of organs, and describe it as forming granules of light yellow pigment which do not give the iron reaction, but stain with Nile blue, fuchsin, and safranin. It is soluble in alkalis and bleaches with hydrogen peroxide. Mallory¹⁷ has further found it in normal organs, in the liver, pancreas, heart and kidneys. It was found in adults above the age of 45 and was always associated with hemosiderin. He found it associated with hemoglobin and hemosiderin in the wall of a chocolate cyst of the ovary and has concluded that hemofuscin is an intermediate product between the two.

Having ruled out the possibility that this "wear-and-tear" pigment is a lipochrome, after the development of methods by which the latter pigment could be rather definitely identified, it seemed necessary to apply these and other known methods to an examination of this mysterious pigment and to correlate, if possible, the results of other workers. Therefore a review of the properties by which all the known pigments are usually differentiated was made with the following result:

1. *Melanin*: Blackened with silver nitrate; precursor positive to dopa; does not stain with fat stains; insoluble; bleaches with oxidizing agents.

2. *Derivatives of Hemoglobin*:

- (a) *Hemosiderin*: Gives the iron reaction (Prussian blue with potassium ferrocyanide and acid); not bleached.
- (b) *Hematoidin*: Crystalline character; negative reaction to stains; no iron; soluble.
- (c) *Hemofuscin* (Mallory): Stains with basic fuchsin; no iron; bleached with hydrogen peroxide.

3. *Lipochromes*: Stain with fat stains; soluble in fat solvents; are easily bleached.

4. *Lipofuscin* (Borst): Stains irregularly with fat stains; insoluble; negative to silver nitrate; bleached with difficulty.

It was obvious from the start that these properties were by no means specific. For instance, practically all pigments can be bleached, and, while most pigments may be tinged with fat stains, I have previously found that lipochrome, in particular, does not take these stains. Consequently, a revision of the methods used had to be made and a technique developed, the details of which are as follows:

TECHNICAL PROCEDURE

1. *Unstained Sections*: Formalin-fixed, cut on the freezing microtome.

2. *Fat Stains*: The staining of formalin-fixed frozen sections with acetone-alcohol solution of Scharlach R, and aqueous solution of Nile blue sulfate.

3. *The Dopa Reaction*: This is said by Bloch to be specific for the precursor of melanin in cells, revealing it as brown or black pigment in those cells which have a potential melanin-producing property. It does not affect, or only slightly darkens, fully formed melanin. Briefly, the technique is as follows: A 1:1000 solution of 3-4 dioxy-phenylalanine in triple distilled water which has been freshly boiled and which has a pH of 7.3 to 7.4, is made immediately before using. A buffer solution (an appropriate mixture of primary and secondary sodium phosphate) is added in sufficient quantity to keep the pH within the limits mentioned (about 2 cc. per 100 cc.). Fresh unfixed tissues cut on the freezing microtome are placed in this solution for from 16 to 24 hours at room temperature, or for 4 to 6 hours at 37° C. In using the method, sections of skin (I used mostly black guinea pig skin) are placed in the solution with the sections to be studied and examined from time to time for results. All tissues should be removed before greatly discolored and before a precipitate forms. The sections are washed in distilled water, allowed to dry in the air on a slide, cleared with xylol and mounted in balsam. Some sections were counterstained with methyl green-pyronin, as recommended by Bloch.

4. *Silver Nitrate Reaction*: Paraffin sections of formalin-fixed tissues were made at first, using Wright's rapid technique (Mallory and Wright, Ed. 8, p. 447), but considerable precipitate was always

present. Bielchowsky's method gave cleaner tissues, but also usually left some precipitate. Levaditi's method with blocks of tissue gave the most uniform results. These were made in the usual way and the first fifty or so sections discarded. Sections from the deeper layers contained little or no precipitate.

5. *Bleaching Agents*: Ferric chloride, peroxide of hydrogen, or direct sunlight were used to cause bleaching, and the reaction is recorded as positive if fading of the pigments could be caused by any process short of their actual disintegration by chemicals. Ferric chloride was used in saturated solution in 50 per cent alcohol and applied to the section on slides while under occasional observation. Hydrogen peroxide was used in 3 per cent solution into which the sections were placed for from 24 to 90 hours, sometimes changing the solution several times. Blocks of tissue were dried in sunlight before or after treating with dehydrating agents and gross observation made. (This could be applied to adrenal cortex, skin, corpus luteum, seminal vesicles and testicle.)

6. *Fat Solvents*: Acetone and chloroform were used, the chloroform after dehydration with acetone. The usual process of making paraffin sections was not relied upon to dissolve out fat and lipochrome, as frequently some of the less soluble lipoids, such as cholesterol, are left in the tissues and give false reactions with fat and basic fuchsin stains. Chloroform alone, without previous dehydration of the tissues, will not extract all the lipochrome.

7. *Alcoholic Potassium Hydroxide*: Used according to a method previously reported: 10 per cent potassium hydroxide in 70 per cent alcohol plus an equal amount of 4 per cent formaldehyde solution. The tissues are placed in this solution for from 40 minutes to 2 hours, dried on a slide, and observed directly or after mounting in glycerine. This solution will dissolve the tissue if prolonged, and one must vary the time according to the tissue under investigation. The pigment, if lipochrome, collects in small masses, or precipitates as crystals which become visible in the microscope. Melanin and hemoglobinogenous pigments are not affected.

8. *Basic Fuchsin*: This is said by Mallory to stain hemofuscin. It also stains other substances, such as lipoids and the cytoplasmic granules of tissue mast cells. It does not stain melanin. The stain was used 0.5 per cent in 50 per cent alcoholic solution upon tissues after thorough extraction with fat solvents, and most sections were

differentiated in 95 per cent alcohol until practically colorless. The pigment which stained by this method could not be decolorized by alcohol.

9. *Iron Reaction*: Mallory's method with ammonium sulphhydrate followed by potassium ferricyanide and acetic acid was used. The sections were counterstained with basic fuchsin, and differentiated in 95 per cent alcohol.

10. *Chemical Examination for Lipochrome*: Organs were dissolved and saponified in alcoholic potassium hydroxide, dehydrated, and extracted with petroleum ether after a method previously described. In some cases the resultant pigment was identified by spectroscopic examination.

TISSUES EXAMINED

The source of the tissue, and the pertinent histories are as follows:

I. *Heart Muscle*:

1. From a six weeks' old infant, dying following an operation for pyloric stenosis; very little pigment present.
2. From a 44 year old woman with nephritis and a large heart; abundant pigment present.
3. From a man of 79, death from peritonitis following a ruptured duodenal ulcer. Weight of heart 280 gm. A fair sample of brown atrophy.
4. From a man of 81 dying of arteriosclerosis and bronchopneumonia. Heart normal in weight; abundant pigment.

II. *Liver*: From the four cases named above, and several from guinea pigs. The latter reacted essentially the same as the human livers.

III. *Intestine*: From the men of 79 and 81 years.

IV. *Spleen*: From the four cases mentioned above and from a case of lobar pneumonia in a young man.

V. *Seminal Vesicle and Prostate*: From the old men, and a man of 21 with pneumonia.

VI. *Adrenal Glands*: Eight from various sources, all essentially normal.

VII. *Corpus Luteum*: From surgical specimens at the Peter Bent Brigham Hospital.

VIII. *Skin*: From several of the adults mentioned above, from a 3 months' old infant and from a black guinea pig. All normal tissues.

IX. *Testicle*: From two old men.

X. *Carotin Lesion*: A granulomatous lesion which followed the injection of pure carotin into the peritoneal cavity of a guinea pig.

XI. *Old Hemorrhage*: A section of ovary containing a hemorrhagic cyst, the wall of which was formed by granulation tissue and endothelial cells. A great deal of pigment was present in cells and interstitial tissues.

RESULTS

Heart: In unstained sections the characteristic pigment is noted in granular yellow to brown masses at each end of the nucleus, or irregularly scattered throughout the muscle. It becomes rusty-red with Scharlach R., green with Nile blue sulfate. The color is deepened by silver nitrate and after prolonged treatment some of the dense masses appear almost black. It can be bleached after extraction with chloroform and treatment with hydrogen peroxide; is harder to bleach with ferric chloride alone. It is negative to fat solvents, alcoholic potassium hydroxide and dopa. No lipochrome could be extracted. Some of the pigment gives the iron reaction; most of it stains intensely red with basic fuchsin, but some is only tinged with the dye.

Liver: Unstained: yellow and brown pigment in liver cells; small amount in Kupffer cells. Scharlach R stained some pigment red, some only a rusty yellow. Blue and green pigment present with Nile blue. Much of the pigment is blackened by silver nitrate; some only moderately darkened. Most of it can be bleached. It is negative to dopa, fat solvents and alcoholic potassium hydroxide, except that some seems to have been dissolved. Much of it is stained by basic fuchsin but some is only tinged by this dye. Some gives the iron reaction. Lipochrome could be extracted (carotin 1 to 6 mg. per cent).

Spleen, Intestine, Seminal Vesicles, Prostate, Testicle: The reactions in these organs were essentially the same as in the heart and liver. In all the pigment was tinged red with Scharlach R, and most often green, that is to say, not stained, with Nile blue sulfate. The pigment could be darkened by silver nitrate and where dense masses of it occurred it appeared black. Most of the pigment could be

bleached out. In all, the dopa reaction, reaction to fat solvents and alcoholic potassium hydroxide were negative, except that sometimes the alkali appeared to dissolve some of the pigment. Two constant results were obtained with basic fuchsin, namely, a deep red-staining substance in droplets and granules, and a more granular material which was only tinged with the dye. In all except the testicle more or less iron-containing pigment could be demonstrated. Only the spleen yielded lipochrome upon extraction (carotin, a trace to 2.1 mg. per cent). The pigment of the interstitial tissue of the seminal vesicles appeared to be the same as in the epithelial cells. Some of this was scraped away, treated with absolute alcohol, in which it was insoluble, then taken up in dilute ammonia water in which it became soluble. This gave a positive guaiac reaction, but contamination with blood could not be excluded, though the tissue had been well washed previous to treatment. In the testicle the pigment was always present in interstitial cells, and nearly always took an intense red stain with fuchsin. A rare blue dot could be found in tissues examined for iron, but the reaction was, on the whole, negative in this organ.

Adrenal Cortex, Corpus Luteum, Carotin Lesion: These tissues gave the same reactions. Unstained, the pigment of the adrenal cortex and corpus luteum occurs in such fine particles that it cannot be resolved in the microscope. The tissue appears uniformly yellow, and all the pigment is obscured when fat stains are used. It is dissolved out by chloroform after dehydration with acetone, is readily bleached with ferric chloride and potassium hydroxide, and when treated with alcoholic potassium hydroxide and formalin the pigment collects in small aggregates and in visible yellow to reddish yellow crystals. It is negative to dopa. Fuchsin stains the fat in which it is dissolved, but after treatment with fat solvents neither fat nor pigment remains to be stained. Silver nitrate precipitates in the cytoplasm of the fat-laden cells, but in the carotin lesion where large masses of lipochrome occurred, a darkening of the pigment occurred with silver nitrate. The pigment, upon extraction, proved to be carotin (adrenals: carotin, 4.25 to 15.6 mg. per cent; corpus luteum: carotin, 4.1 mg. per cent; this also contained an alcohol-soluble pigment, possibly xanthophyll). No pigment could be demonstrated or extracted from the adrenals of a 3 months' old infant.

Adrenal Medulla: The pigment here is brown in unstained sections, takes a rusty tinge with Scharlach R, and green with Nile blue. It becomes intensely black with silver nitrate and can be bleached. It could not be seen that dopa increased the amount of pigment present, though it darkened the existing pigment somewhat. It was negative to fat solvents, alcoholic potassium hydroxide, basic fuchsin and the iron reaction. Chemical examination could not be made.

Skin: Yellow to brown pigment was present in many of the basal cells in the unstained section. In addition a yellow crystalline pigment could be seen in some sections in the cornified outer layer, quite sparse in most specimens and absent in some. The two pigments gave different reactions. The first, in the basal cells, was not stained by Scharlach R, but became green with Nile blue. It was intensely blackened by silver nitrate, was negative to fat solvents, alcoholic potassium hydroxide and basic fuchsin, and did not give the iron reaction. Dopa increased the amount of pigment present and slightly darkened the existing pigment. The second pigment was tinged with Scharlach R, not stained with Nile blue, was darkened slightly with silver nitrate, and unaffected by dopa. It was more easily bleached than the first pigment but both bleached finally. Most of the outer pigment was soluble in fat solvents, negative to basic fuchsin and the iron reaction, and, being already crystalline, it was not affected by alcoholic potassium hydroxide. These were obviously different pigments, the deeper being melanin, the outer, lipochrome. (Some of the outer pigment might also have been melanin, as all was not dissolved out.)

Old Hemorrhage: Abundant yellow and light brown pigment was present in the connective tissue and endothelial cells surrounding the cyst. Some of this was tinged red with Scharlach R, and green with Nile blue. Some of the pigment was definitely blackened with silver nitrate. It was unaffected by dopa, not soluble in fat solvents, but some seemed to have been dissolved out by alcoholic potassium hydroxide. Much of the pigment stained intensely red with fuchsin and about half gave the iron reaction. Some yellow pigment was tinged red with fuchsin and some was not stained at all. Chemical examination was not made.

DISCUSSION

It seems somewhat difficult to bring order out of the chaotic state in which one is left after a study of the varying opinions recorded above. It is obvious that too much reliance has been placed upon what are considered to be specific histologic reactions. There are a few points, however, upon which almost universal agreement exists, and if one concedes that silver nitrate, a notoriously unreliable agent, does upon occasion darken or even blacken other pigments besides melanin, a correlation can be made which is compatible with most observed facts and with some opinions. If silver nitrate blackens melanin only, then one must conclude that this pigment is normally and usually present in the liver, spleen and hemorrhagic foci, and develops in granulomatous lesions (such as those produced by the injection of carotin), a conclusion which seems entirely unreasonable. In fact, no pigment seems to be completely unaffected by this salt, all of them showing the same brownish tinge which intercellular substances exhibit. If the pigments occur in densely packed masses the density of color is increased proportionately and the appearance of blackening becomes an optical rather than an actual effect. This is probably the case in the liver and spleen where comparatively large amounts of pigments are normally found.

All pigments except hemosiderin can be bleached, lipochrome the most readily, and practically all are lightly stained with Scharlach R. Nile blue sulfate, as pointed out by Hueck, gives a greenish appearance to pigments, not because of actual staining, but because of the mixture of yellow pigment and blue dye. These dyes, and others, seem merely to be adsorbed to the surfaces of the pigments.

The dopa reaction is not expected to differentiate pigments once fully formed. The results recorded here are therefore not conclusive, but do confirm other work in that no substance was found in heart muscle, liver and other organs, except the skin, and possibly the adrenal medulla, which was capable of forming melanin from dioxyphenylalanine.

Fat solvents, properly used, effectively remove true lipochrome and alcoholic potassium hydroxide affects this pigment only, causing it to aggregate and crystalise from its usually dispersed particles.

The two most constant reactions have been those produced with basic fuchsin stain and the test for iron. Two reactions to fuchsin

were present; (1), an intense red produced in a substance which appeared frequently as droplets rather than granules, and (2), a red tinge in definitely granular pigment. In the hemorrhagic focus one can hardly escape the conclusion that these substances represent stages in the transformation of hemoglobin into hemosiderin, a conclusion previously reached by Mallory. The intense red pigment seems to be a semiliquid substance which by condensation becomes granular and so gradually loses its affinity for the fuchsin stain. If this is true for the hemorrhagic focus, it seems likely that it is also true in other places where a similar association of pigments is found.

This brown pigment (hemofuscin, Mallory; lipofuscin, Borst; fetthältige abnutzungspigment, Sehrt; abbaupigment, Lubarsch; alterspigment, Oberndorfer;¹⁸ lipomelanin, Kutschera-Aichbergen;¹⁹ sometimes lipochrome in American literature) is constantly associated with hemosiderin in the normal heart, liver, spleen, smooth muscle of the intestine, seminal vesicles and prostate; is present, with hemosiderin, in increased amount in brown atrophy of the heart and liver; increases proportionately with hemosiderin in all these organs and others in hemochromatosis (Mallory); and is always associated with hemosiderin in local hemochromatosis (pseudomelanosis, ochronosis) of the large intestine (Lubarsch). Hemosiderin was present in very small amount in the testicle, and not at all in the heart of the 6 weeks' old infant, though pigment which stained with fuchsin was present in both these organs.

A word of caution is needed here. One assumes, without justification perhaps, that most material of a granular nature which is shown to contain iron is hemosiderin (that is, iron derived from hemoglobin). Where this substance is found associated with hemorrhage, the conclusion that it comes from hemoglobin seems obvious. But Sprunt, Colwell and Hagan,²⁰ have apparently shown that iron may be derived from other proteins during autolysis, and that an iron-containing pigment is not necessarily a product of hemoglobin decomposition. Hueck has said that hemosiderin is, in fact, an inorganic iron compound by the time it is recognizable in tissues, and so has no property by which its origin can be traced. The only point one can make here is that, whatever the source, it seems to be the same for both hemofuscin and its iron-containing satellite.

True lipochrome (carotin) can be demonstrated histologically in the adrenal cortex, the corpus luteum, in atheromatous plaques of

the aorta and the skin. It cannot be differentiated from other pigments in the liver and spleen, although it was shown to be present in these organs by chemical examination. Also, because of the nature of the tissue, it is not demonstrable histologically in fat, but can be extracted from adipose tissue. It is to be noted that lipochrome was not present in any of the tissues of the infants studied.

This pigment should offer no morphological difficulty. Only in the outer layers of the skin can it be seen as a granular pigment. In all other places it occurs in solution in lipoids, giving the tissue a yellow coloration, but is not visible as a particulate substance by the microscope. The granules in the skin are probably formed by condensation and precipitation in the outer layers as the epidermal cells are pushed outward and become keratinized. It is difficult to find the pigment in this tissue probably because it is actually scant in normal skin, and because a large part of it becomes oxidized and is then invisible.

Melanin could be demonstrated beyond a reasonable doubt only in the skin, although a definite effort to locate this pigment in all tissues where it might occur was not made. The pigment of the adrenal medulla gave all the reactions of melanin, and none of those which have been found to distinguish other pigments. If this work and that of Bloch and Masson are conclusive, they show that melanin is a constituent of certain special cells only, and cannot be regarded as the product of degenerative processes in tissues.

CONCLUSIONS

A revision of the characteristics of these several pigments must be made. So, we have:

1. *Melanin*: A brown pigment occurring in certain cells of ectodermal or nervous tissue origin (ectodermal melanoblasts) and, in man, in what are essentially cell rests, in cells of mesodermal origin (mesodermal melanoblasts) which form Mongolian spots and certain blue nevi. In addition it may occur in phagocytic cells (melanophores, chromatophores) in the corium. There is no definite evidence that true melanin occurs elsewhere. The pigment is intensely blackened by silver nitrate and is revealed, in cells which are capable of forming it, by the dopa reaction. It is insoluble in acid and alkaline solutions, and in fat solvents. In common with all other pig-

ments except hemosiderin it is bleached by oxidizing agents. It occurs pathologically in melanotic tumors.

2. *Hemosiderin*: An iron-containing pigment, insoluble, may be darkened with silver nitrate, does not bleach, stains irregularly with fat stain, is lightly stained with basic fuchsin. It occurs in phagocytic cells and intercellular tissues (after degeneration of the cells which originally contained it), normally in the spleen and liver (epithelial and Kupffer cells), and in advancing age in the muscle of the heart, intestine, seminal vesicles, prostate and blood vessels. It is increased in the heart and liver in brown atrophy of these organs; and in these and many other organs in hemochromatosis; is present in the mucosa, submucosa and muscle of the intestine in pseudomelanosis, or local hemochromatosis. It is always present in the vicinity of old hemorrhages.

3. *Hematoidin*: Yellow crystalline or semiliquid (dissolved in tissue juices), soluble, is darkened with silver nitrate, bleaches readily, contains no iron and does not stain with fat or basic dyes.

4. *Hemofuscin*: Yellow or brown semiliquid or crystalline; insoluble in fat solvents, partly soluble in alkali; does not give the iron reaction, but darkens with silver nitrate; stains lightly with fat stains (by adsorption); in early stage of formation it stains intensely with basic fuchsin, but as it becomes crystalline, it stains only lightly with this stain, and bleaches less readily than hematoidin, melanin and lipochrome. *Hemofuscin is present in all places and under the same conditions as hemosiderin*, in usually greater amount. It, with hemosiderin, constitutes the "wear-and-tear" pigment of the body, and is probably a product of the metabolism of hemoglobin, both from blood and muscles.

5. *Lipochrome (Carotin, Xanthophyll)*: A diffuse, finely dispersed yellow pigment, associated with fat. It does not stain with fat stains, is soluble in fat solvents, bleaches readily and darkens with silver nitrate (when crystalline). It is aggregated, or becomes crystalline, when treated with alcoholic potassium hydroxide. It occurs naturally as crystals only in the outer layers of the skin. It is present normally in the adrenal cortex, corpus luteum, liver, spleen, fat and skin; increases in these tissues with increased ingestion of food, or when, for some reason, lipemia is present. It occurs in pathologic processes in atheromatous plaques of arteries, and in xanthomas. It is not present in the tissues of infants.

There are therefore two pigments which accumulate in the body tissues in advancing age, and seem to be increased in cachectic conditions. Only one of these, however, (hemofuscin, which apparently slowly becomes hemosiderin), can be regarded as a "wear-and-tear" pigment, the result of slow or rapid disintegration of a tissue protein. (The fact that this pigment is present in the heart of infants is dismissed by Rössle ²¹ with the statement that "Man is old before he is born.") When lipochrome is present in increased amount it is due to increased ingestion, or to the absorption of the fat containing it. In the latter condition, no more lipochrome is present than formerly, but the proportion to the amount of remaining solvent is greater.

SUMMARY

A review of the characters which are said to differentiate the various pigments of the body was undertaken in conjunction with the chemical examination of certain organs for lipochrome (carotin and xanthophyll). Tissue from the normal heart, liver, spleen, skin, intestine, seminal vesicles, prostate and testicle, as well as a lesion produced by carotin injection in a guinea pig and an old hemorrhagic focus, were treated in identical ways histologically, and where feasible, chemically. Two groups of pigments were found to accumulate in the body with age, namely, lipochrome and hemofuscin (with hemosiderin). The former of these is exogenous in origin; the latter constitutes the real "wear-and-tear" pigment, and possibly is derived from muscle hemoglobin.

From the results of this inquiry the pigments present in the various tissues studied seem to be:

1. *Skin*: Melanin and lipochrome; also, in hemochromatosis; hemofuscin and hemosiderin.

2. *Fat*: Lipochrome.

3. *Heart, Intestinal Muscle, Seminal Vesicles, Testicles, Prostate*: Hemofuscin and hemosiderin. Increased in old age, brown atrophy and hemochromatosis.

4. *Liver and Spleen*: Hemofuscin and hemosiderin besides bile pigment. Increased in old age, brown atrophy and hemochromatosis.

5. *Adrenal Cortex*: Lipochrome.

6. *Adrenal Medulla*: Probably melanin.

7. *Corpus Luteum*: Lipochrome.

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SOME POINTS ON THE MECHANISM OF FILTRATION BY THE SPLEEN *

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Among the many and varied functions attributed to the spleen, that which stands out most prominently is its ability to filter out from the flowing blood stream foreign particulate matter such as broken down red and white blood cells, platelets, carbon and iron pigment, and other materials. This function was recognized by Ponfick in 1869,^{1, 2} who found that it held back not only degenerating red blood cells but also other foreign substances. He spoke of this function as being "spodogeneous" in nature. Frey,³ and Rautman,⁴ confirmed these observations that the spleen possesses the function of filtration by finding fewer red cells in the splenic vein than in the artery. This removal of red blood cells by the spleen they found was related to the change in the osmotic resistance of the red cells. When this resistance was increased by phenylhydrazin the number of cells in the vein equalled that in the artery, while on the other hand, if it was lowered by ether the difference in number became very much greater. Frey also showed that foreign red blood cells introduced into the splenic artery were held back in the pulp. Opie⁵ got similar results by the transfusion of blood from one animal to another. Bedson⁶ has shown also that the spleen regulates the number of platelets in the circulation, taking up and destroying those which are of no further use. Voorhoeve⁷ regarded the spleen as a filter for all abnormal materials present in the blood.

Considerable experimental work has also been done to prove this filtering function of the spleen on the circulation. Wyssokowitsch,⁸ the first to make intravenous injections of bacteria and to note their distribution, found that many of them were caught in the spleen. Bouffard⁹ and Goldman,¹⁰ with intravenous injections of dyes, found that in part they were deposited in the spleen. Duhamel,¹¹ using colloidal solutions of various metals and Foot,¹² Tait and

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McCartney,¹³ and Nagao,¹⁴ using India ink for intravenous injections, found considerable deposits in the spleen. Drinker and Shaw,¹⁵ using a suspension of manganese dioxide, got similar results. These observations and experiments tend to show that the spleen is somewhat of the nature of a filter for the blood stream, being able to remove from it particulate matter of a foreign nature.

The ordinary conception of a filter is a device for mechanically straining out particulate matter from a fluid menstrum. While this may be true in a general way, the mechanism by which filtration takes place is often very complex. There are a number of variable factors which will determine whether or not a given substance will be strained out. The size of the particle in relation to the size of the pores of the filter is an important one. If the particles are larger than the pores, the process of course is quite simple, being merely a mechanical separation of the particles from the menstrum. On the other hand, in the case of minute particles of a colloidal solution and even of bacterial suspensions, when such filters as the Berkefeld or Chamberlain are used, mechanical separation no longer plays the important part. These filters were long thought to separate the bacteria from their suspending fluids, by virtue of the small diameter of the pores. Bechhold,¹⁶ for instance, in 1908, calculated the mean diameter of the larger pores of a new Chamberlain "F" filter, to vary between 0.23 and 0.41 microns. Mudd,¹⁷ using Bechhold's formula, found the average diameter of Berkefeld filters of the "V" type to be 0.38 microns, the "N" type to be 0.45 microns, and the "W" type to be 0.43 microns. As most bacteria are larger than these diameters the process of filtration appeared to be mechanical in nature. Bigelow and Bartell,¹⁸ however, in 1909 pointed out an error of one decimal point in Bechhold's formula. The corrected formula showed the pores to be ten times larger. Using the figures given above and allowing for the error in the Bechhold formula the mean diameter of the pores of the Chamberlain "F" filter should have been 2.3 to 4.1 microns and in the case of the Berkefeld filters 3.8 microns for the "V" type, 4.5 microns for the "N" type and 4.3 microns for the "W" type. These diameters are greater than those of most bacteria and should offer no hindrance to their passage.

The work of Mudd¹⁷ on the passage of cultures of *Vibrio percolans* and that of Wolbach, Binger, and Todd,¹⁹ on the passage of certain spirochetes through Berkefeld filters shows that there is no reason

based on mechanical obstruction why bacteria should not pass through. Bulloch and Craw²⁰ found many Berkefeld filters even at atmospheric pressure permeable to bacteria. However, this is not generally true for with good filters most bacteria are held back, there being only a few strains which can consistently be passed through.

Berkefeld and Chamberlain filters, having such large diameters to their pores, obviously do not mechanically separate bacteria from the filtrate. Neither does motility of the bacteria play a part, for Mudd¹⁷ was unable to pass the much more motile organism, *Vibrio comma*, through a filter which previously passed *Vibrio percolans*. As for the mechanism of filtration, Mudd,^{21 22} offered the suggestion that it was dependent upon electrical attraction between the bacteria or particulate matter and the wall of the filter. He described the surface of the pores of a Berkefeld filter as the site of an "electrical potential difference," a helmholtz double layer. The wall of the filter, he believed, carried a negative charge and the adjacent liquid a positive charge. He predicted and found that positively charged particles in suspension were adsorbed and retained by the filter while negatively charged particles passed through. *Vibrio percolans* were found to be negatively charged and as the wall of the filter carries a negative charge, the bacteria were repelled and passed through. This conception was further substantiated by Kramer²³ who made a filter that was oppositely charged. He made it with commercial plaster of Paris. The positive charge on the walls of its pores depended upon the presence of calcium carbonate in the mixture and was further enhanced by adding up to 25 per cent of magnesium oxide, calcined at 1300° C. He now possessed a filter of opposite charge to that of the series of siliceous filters such as the Berkefeld and Chamberlain. He observed that with this he could remove from the menstrum all colloids and suspensions which passed through Berkefeld filters and *vice versa* all substances which passed through the plaster of Paris filter were readily held back by the siliceous filters. He then constructed a combined filter, using a Berkefeld filter for a core and a plaster of Paris filter for a cortex. Colloids both positively and negatively charged were removed by this combination filter.

STRUCTURE OF THE SPLEEN

The internal structure of the spleen is such as to suggest that it is primarily a filter. It is essentially a spongy mass of reticulo-endothelial cells belonging to the system as described by Aschoff.²⁴ It is surrounded by a capsule and supported by a trabecular framework of smooth muscle and elastic fibers with a connective tissue stroma. This framework serves to control the flow of blood through the organ. The blood is widely distributed by an arterial system which divides and terminates in end capillaries. The latter have been shown by the author²⁵ to open out and discharge their contents into a vast cavernous network of pulp spaces formed by the above mentioned reticulo-endothelial cells as they stretch across the intervening spaces of the trabecular framework. The circulation is continued into the venous channels through slit-like stomata in the walls of the veins. The circulation is therefore open. The end capillaries are unique in that toward the ends they are ensheathed in a condensed mass of pulp cells called ellipsoids. These will be shown later to play an important part in the mechanism of filtration.

The spleen differs from ordinary filters, such as the Berkefeld, in the fact that the size of its pores varies with relaxation and contraction of its muscular system and the flow of fluids through its pores is more or less intermittent, being controlled by this same muscular system. It is similar however in the fact that the suspended material is brought directly in contact with the walls of the pores, namely, the pulp ellipsoid cells. This is brought about first by the devious courses taken by the blood through the maze of pulp spaces and secondly by the stagnation of the blood during periods of relaxation of the muscular system. The chance of contact of pulp cell and particle has been dealt with from a theoretical standpoint by McKendrick²⁶ and experimentally by Fenn.²⁷ The latter has shown that if a suspension of cells which have a diameter "C" and velocity (under gravity) V_c and of particles of a diameter "P" is allowed to settle in a test tube the chances of collision "R" between them will be proportional to the velocity of the particle to the cell and to the square of the sum of their diameters or $R = (V_p - V_c) (C + P)^2$.

In the case of the spleen, however, the particles alone are in suspension, the filtering cells (pulp and ellipsoid cells) being fixed and having no velocity. V_c therefore is eliminated. The above formula

is also based on the supposition that the cells and particles are spherical in outline. This represents the minimum surface area for a given mass. If the pulp cells were spherical in outline the chances of collision "R" between foreign particles in the flowing blood through the spleen and the pulp cells would be proportional to the velocity of the blood to the square of the sum of the diameters of the particles and of the pulp cells. The surface area of the pulp cells, however, is tremendously increased by the fact that their cytoplasm is stretched out into long filamentous processes. The chances of contact of particles in the flowing blood with pulp cells is thereby considerably increased over that represented by the formula.

Having in mind the mechanism of filtration in Berkefeld and other filters a number of experiments were carried out to answer the following questions: (1) Does filtration occur in isolated as well as in normal spleens? (2) Can the filtrate be dislodged? (3) Is filtration in the spleen mechanical in nature? (4) Is filtration in the spleen dependent upon living cells? (5) Is filtration in the spleen electro-physical in nature?

EXPERIMENTS

Sheep, dog, and cat spleens were used. The filtrating fluid was either perfused through the freshly isolated spleen or injected intravenously and the spleen removed later. As substances for filtration India ink, carmine, acid and basic dyes, and colloid solutions of copper, platinum, and silver were used.

With freshly isolated spleens the anastomotic circulation was tied off and canulae inserted into artery and veins. It was found advisable to suspend the spleen in a basin of warm water while the injections were being carried on. Following, and sometimes also before, they were perfused with either distilled water or normal saline solution. The injections into the artery were made with a pressure varying between 60 mm. and 150 mm. Hg and a back pressure in the vein of 10" of water. These pressures were found sufficient to distend the spleen to capacity without in any way damaging its delicate network of cells. To provide for a proper flow it was found absolutely essential to eliminate all bubbles of air from the filtering and perfusing fluids, otherwise they acted as emboli and blocked the

circulation. If sufficient pressure was applied to dislodge them they caused gross lacerations of the delicate pulp tissue.

The first series of experiments were done to determine whether filtration occurred in freshly isolated spleens as well as *in vivo*. Fresh sheep, cat, and dog spleens were isolated, anastomotic circulation tied off, and the canulae inserted into the vein and artery, and the whole organ suspended in warm water. Ten cc. of a 1 per cent solution of Higgins' India ink was injected into the artery. It was then perfused with warm normal saline at a pressure of 100 to 150 mm. Hg until the return fluid from the vein was clear. Twenty per cent formalin was perfused in the same manner until the spleen was thoroughly bathed with the fixative. While in the distended state ligatures were applied to the artery and vein. Gross and microscopic sections showed that filtration had occurred, for adherent to the filamentous processes of the pulp cells were large deposits of India ink. Perfusions of distilled water, both before and after the India ink injections, failed to alter the filtering process. Intravenous injections of similar solutions showed again that the spleen possessed the ability to separate India ink particles from the blood for considerable deposits were found on the ellipsoid and pulp cells. These experiments further demonstrated that even with prolonged perfusion the ink particles could not be dislodged by normal saline or distilled water. It was found advisable to make the perfusions with distilled water as the saline solution caused the fine particles of ink to conglomerate and settle out in small masses.

The mechanical factor in the filtration would seem to be eliminated by the fact that the pulp spaces are much larger than such colloidal particles as India ink. The pulp spaces have been shown by the author²⁴ to vary between 0.005 to 0.02 mm. in diameter. However, to substantiate this, freshly isolated sheep, cat, and dog spleens were perfused with warm normal saline at a pressure head of 100 mm. Hg. in the artery and a back pressure of 10" of water in the vein. This caused the spleen slowly to expand to its capacity, and as has been shown before to dilate all the pulp spaces to their full diameter without rupturing the pulp cells. The vessels and pulp spaces being dilated to capacity a 1 per cent solution of India ink was slowly introduced into the perfusing fluid, and perfusion continued until return flow. After these injections the spleens were perfused with 20 per cent formalin until thoroughly impregnated

with the fixative. Gross sections showed that filtration was very effective, the pulp tissues being quite dark gray in color. Microscopically, the vessels and pulp spaces were found to be dilated to capacity and free from any particles of ink. The filamentous processes of the pulp cells however, were covered with fine deposits of ink particles. It is quite apparent therefore that mechanical separation by virtue of the size of the pores in relation to the size of the particles of ink is eliminated.

By the foregoing experiments it has been shown that the spleen is able to filter out India ink particles from the blood plasma, normal saline solutions and distilled water. The distilled water must have interfered with the vitality of the pulp cells. This, however, did not hinder the process of filtration. To prove more definitely that filtration by the spleen is not dependent upon the vitality of its cells, two freshly isolated sheep spleens were perfused, first with normal saline, then with 100 cc. of a 1 per cent solution of sodium cyanide. Perfusion with normal saline was continued for a few minutes, then 40 cc. of 1 per cent India ink solution was introduced. Filtration appeared to occur just as readily as in the previous experiment. This was verified by microscopic examination. Large deposits of the ink particles were found adherent to the filamentous processes of the pulp cells. Filtration by the spleen, therefore, is not dependent upon the vitality of its cells.

Having in mind the factors which determine filtration in Berkefeld and plaster of Paris filters, it was thought advisable to test these on the spleen. While up to the present only a few experiments have been made, they seem to indicate that the mechanism of filtration is selective in character and dependent upon the electrical charge of the suspended particles. Colloidal solutions (Bredig) of platinum, silver, and copper were used. Colloidal particles of platinum and silver are negatively charged, while those of copper are positively charged. Perfusion experiments with isolated spleens were not found to be very satisfactory. This was particularly true when normal saline was used. The colloids were precipitated almost immediately in the presence of the salt and were deposited upon the walls of the larger vessels.

The results obtained by first perfusing with distilled water were somewhat better. A freshly isolated spleen was perfused first with distilled water then injected through its artery with 175 cc. of a

colloidal silver solution (negative) and perfusion continued for ten minutes. The second spleen after first perfusing with distilled water was injected with 110 cc. of colloidal platinum solution (negative) and followed by further perfusion with distilled water, as in the first case. The third spleen was perfused in the same manner as the first and second, but 325 cc. of a colloidal copper solution (positive) was injected into the artery and perfusion continued. After fixation, sectioning and staining it was found that the first and second spleens respectively had filtered out the platinum and silver. Particles were found adherent to the pulp cells as seen in the illustrations. In the case of the third spleen, no evidence of copper deposits could be found, either directly or after Perle's reaction. This latter reaction should show the copper as a brown precipitate.

In order to overcome the tendency of these colloids to precipitate on to the walls of the large vessels and to take advantage of the protective colloids of the blood plasma, intravenous injections were tried.

A dog weighing 3.5 kg. under an anesthetic during a period of nineteen minutes was injected intravenously with 325 cc. of a colloidal copper solution (positive). The dog died immediately after the injection. Blocks were taken from various areas of the spleen and closely examined for traces of copper deposits, but none was found.

A cat weighing approximately 2 kg. was then anesthetized and during a period of seven minutes was injected intravenously with 60 cc. of a colloidal platinum solution (negative). After nineteen minutes from the commencement of the injection the animal was killed and the spleen fixed and sectioned as in the previous case. Microscopically, fine deposits of the platinum particles could be seen upon the cells of practically all the ellipsoids.

COMMENT

While it has long been known that the spleen filters out foreign materials from the blood stream, the mechanism of this process has not been clearly demonstrated. In the case of Berkefeld and similar filters it was formerly thought to be a mechanical separation by virtue of the small diameter of their pores. Bechhold's¹⁶ formula for estimating these diameters would seem to substantiate this view but more recent experimental work and the calculations of Bigelow

and Bartell¹⁸ showed that the process could not be one of a mechanical nature. Mudd has suggested that filtration by Berkefeld filters is dependent upon adsorption. He believed the walls of the pores to be the site of an electrical charge and to adsorb all particulate matter carrying an opposite charge. The work of Kramer would further substantiate this view.

I have shown that in the case of the spleen, either isolated or *in vivo*, the possibility of mechanical separation of foreign particles from the flowing blood is almost impossible because of the relatively large size of the pulp spaces compared with that of the particles.

The process is not a vital one as filtration occurs just as readily in the isolated spleen whose cells have been rendered functionless by cyanide as in the living spleen.

The mechanism of filtration by the spleen is apparently the same as that of such filters as the Berkefeld, Chamberlain, and plaster of Paris filters. It is one of adsorption of the particle to the wall of the pores or in the case of the spleen it is an adsorption of the foreign particles to the pulp cells. This is a non-vital process and is dependent upon the relation of the electrical charge of the suspended particle to the electrical charge of the surface of the pulp cells. When the charges are relatively the same the particles and cells repel each other and the particles pass through the spleen. When the charges are opposite, the particles and cells are attracted and the adsorption occurs. Filtration is selective in character and dependent upon the electrical charge of the suspended particles. Of course the further process of phagocytosis is another problem and brings in new factors not dealt with in this study.

The work of Duhamel,¹¹ on the intravenous injections of various colloidal solutions and the analysis of the organs for their recovery, would seem to substantiate this theory of selective filtration. He gave intravenous injections of colloidal silver, platinum, and copper, as well as other colloidal solutions. The exact amount of each metal by weight was determined before injection, then a chemical analysis of the various organs was made to determine the distribution. The silver and platinum (negatively charged) deposits were found chiefly in the liver with traces in the spleen. The copper (positively charged) deposits were found chiefly in the blood with slight amounts in the liver and none in the spleen.

Jancso²⁸ has recently made use of this selective affinity of the

reticulo-endothelial system for negatively charged particles to carry certain positively charged colloids to these cells. He used an inert negatively charged colloid like Chinese ink as the vehicle for the drug and by intravenous injections he was able to produce profound changes in this system.

SUMMARY

1. The spleen is essentially a blood filter, filtration occurring equally well in the isolated spleen as in life.
2. The process of filtration is not dependent upon the relative size of the spaces to that of the particles, nor is it dependent upon the vitality of the pulp cells.
3. The process is apparently electro-physical in nature and depends upon the electrical charge of the particle in relation to that of the pulp cell.
4. The adsorbed particles cannot be readily dislodged from the surface of the pulp cells.
5. The spleen filters out negatively charged colloidal particles.

I am indebted to Prof. E. F. Burton, both for the colloid solutions used and for much helpful advice, and to Prof. Oskar Klotz for his help in the supervision of the experiments.

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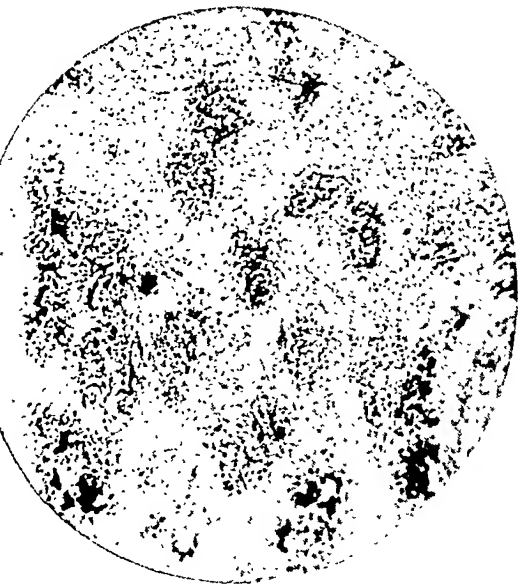
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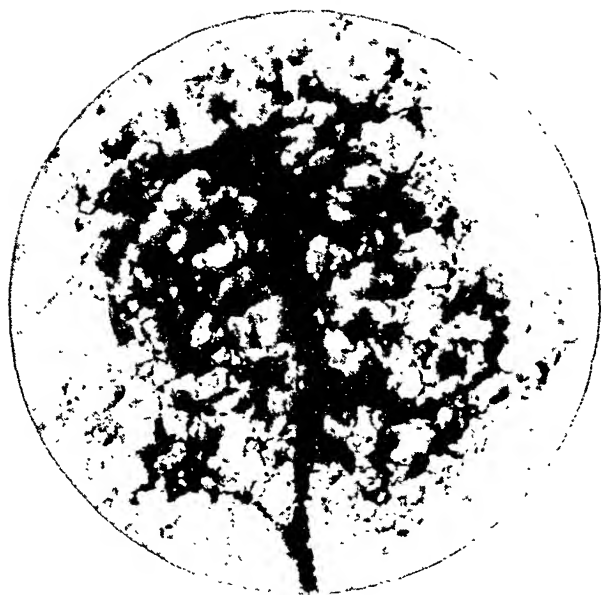
DESCRIPTION OF PLATES

PLATE 74

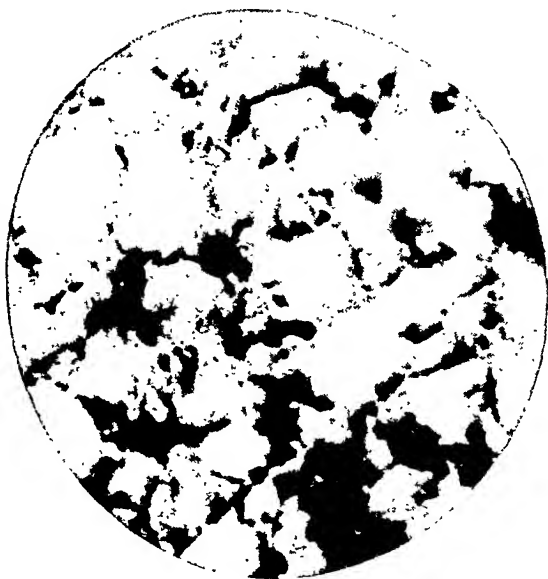
- FIG. 1. Isolated sheep spleen perfused with small quantity of India ink solution. India ink particles adherent to pulp cells about the end capillaries.
- FIG. 2. Higher power showing the India ink filling the end capillary and adherent to the pulp cells about it.
- FIG. 3. High power showing the India ink adherent to the filamentous processes of the pulp cells.
- FIG. 4. Dog spleen, distended to capacity, then perfused with India ink solution. The ink is shown adherent to the walls of the end capillaries and to some of the neighboring pulp cells.



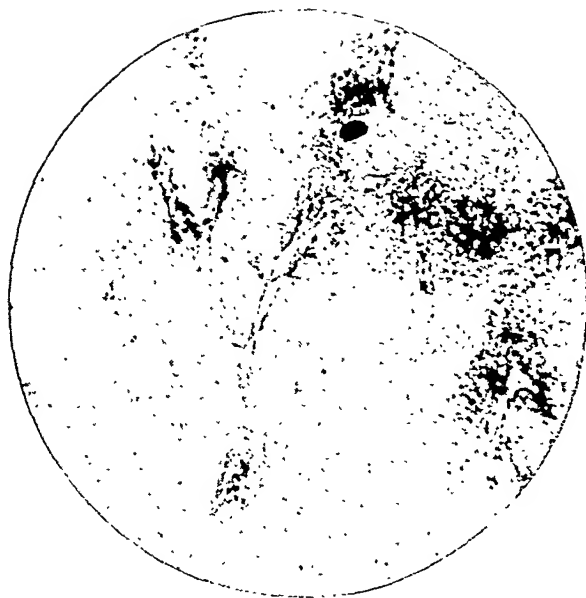
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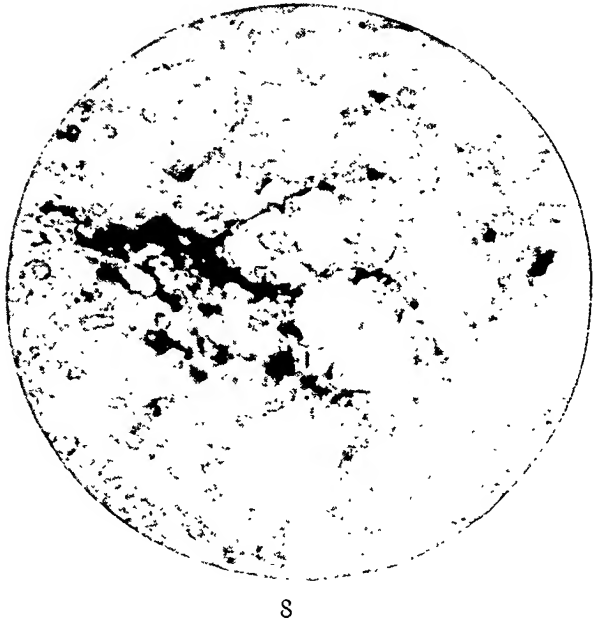
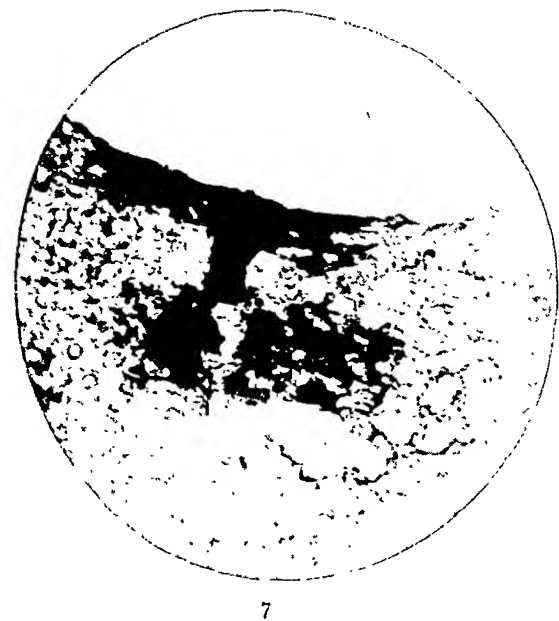
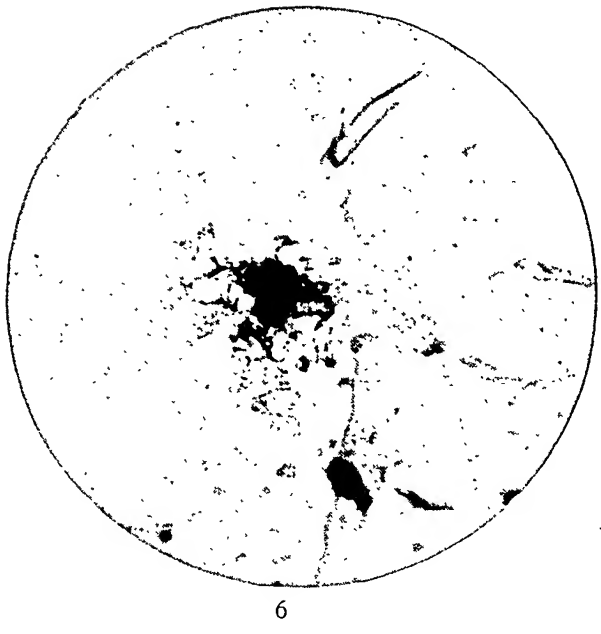
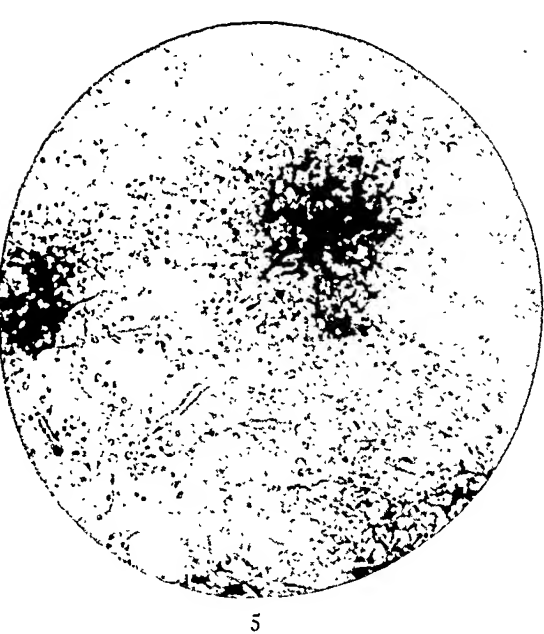
PLATE 75

FIG. 5. Sheep spleen, perfused first with potassium cyanide solution, then India Ink injection. The ink particles have adsorbed to the pulp cells about the end capillaries.

FIG. 6. Colloidal silver (negative charge), solution was perfused through a sheep spleen. The silver particles have adsorbed to the pulp cells.

FIG. 7. Same as Fig. 6.

FIG. 8. Sheep spleen perfused with colloidal platinum (negative charge), solution. Platinum particles have adsorbed to the pulp cells.



HUMAN MERCURIC CHLORIDE POISONING BY INTRAVENOUS INJECTION *

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Cases of mercury poisoning have frequently been observed and reported in which the poisoning followed ingestion, inhalation, absorption from inunction, douches, wound irrigations and subcutaneous or intramuscular injection. Cases terminating fatally following administration by any route other than by the mouth are rare and we have not found any careful study of the pathological changes incident to poisoning by ingestion compared with those in which the mercury entered the blood stream directly.

An exhaustive study of the renal changes in human beings due to ingestion of mercury has been made by Heineke.¹ MacNider² in a series of studies has observed the action of mercury in experimental animals.

A search of the literature has failed to reveal a report of mercury poisoning by the intravenous route in a human being. The almost simultaneous death of four persons following the intravenous injection of massive doses of mercuric chloride has afforded the opportunity to compare the effects of the poison thus administered with the effects when taken by mouth. The four persons received intravenous injections of mercury at the hands of a now defunct institution advertised as a "blood serum clinic." Detectives who arrived at the "clinic" were too late to prevent the destruction of records and of most of the medicaments. Several unbroken ampules labeled 1/12 gr. which assayed 5.386 gr. of mercuric chloride were recovered from the waste can. Ampules of other substances such as normal saline and sodium iodide were also found. Since some of the patients with symptoms of poisoning recovered, although all of those interviewed had received more than one injection, it is supposed that mercury was not given each time but alternated with the injection of other substances. The overdose was attributed to a mistake made

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by the chemist who compounded the preparations. It is certain that in each of the four cases here reported one dose of between 5 and 6 gr. of mercuric chloride was given intravenously.

In 1918 Sansum³ determined the minimal fatal dose of mercuric chloride by intravenous administration in the dog to be quite uniformly 4 mg. per kilogram of body weight. He found larger doses (5 mg. per kilo) necessary to produce anuria, and when animals were once anuric, all attempts to reestablish the flow were futile. Menten⁴ found cloudy swelling in the liver and kidneys of rabbits as soon as five minutes after the intravenous injection of as small amounts as 0.002 mg. According to the lowest calculation our cases received at least 6 mg. per kilogram of body weight.

*CASE I * Clinical History:* M. J., a white male, age 40, was admitted to Cleveland City Hospital on October 19, 1927, complaining of bloody expectoration and difficulty in breathing. Only an incomplete history was obtainable. He had had frequency and dysuria for six years. Late in the summer he had started taking "treatments" at the "blood serum clinic," consisting of intravenous injections. His condition improved, he thought. Three weeks prior to his admission the patient became ill with chills and fever, and later a peritonsillar abscess developed. The only symptom referable to his injections was the onset of anuria two days before his entrance to the hospital. Early on the day of admission the patient was very dyspneic and expectorated bloody sputum. Nothing could be learned about the number of injections he had received.

On examination, he was stuporous, dyspneic and his mouth and lips were covered with blood-stained sputum. Respirations were 10 per minute, the pulse thready, heart-beats 90 per minute, and temperature 35° C. The extremities were cold and moist. The teeth were absent, and the buccal mucosa was covered with bright red blood. Heart, lungs and abdomen were reported negative, but satisfactory examination was impossible. There was a palpable fibrous scar in the anterior urethra.

Laboratory Findings: The blood showed hemoglobin 85 per cent; red blood cells 6,300,000 per c.mm.; leukocytes 48,500 per c.mm.; blood urea 59.1 mg. per 100 cc.; uric acid 11.4 mg. per 100 cc.; creatinine 15 mg. per 100 cc. No urine was obtainable. Blood Wassermann was four plus.

Death occurred four hours and forty minutes after admission. The duration of the intoxication was not known since the date of his last injection could not be learned. The clinical diagnosis was acute nephritis, chronic pyelonephritis, urethral obstruction, uremia, with death due to renal insufficiency.

Autopsy: The heart weighed 300 gm. and was pale but showed no lesions. There was a confluent bronchopneumonia of both lower lobes. The liver weighed 2200 gm. and showed only cloudy swelling grossly. The spleen weighed 175 gm. and appeared hyperplastic.

* For permission to report Cases I and II, I am indebted to Dr. R. W. Scott and Dr. O. Saphir of the Cleveland City Hospital.

The left kidney weighed 300 gm. and was large, soft, pale and friable. The boundaries between cortex and medulla were indistinct. Many small abscesses were seen throughout the kidney substance. The pelvis was dilated. The right kidney weighed 100 gm. and showed the same focal abscesses. The organ was pale, and the pyramids smaller and paler than those of the left kidney. There was a reddish gray mucoid exudate in the bladder near the trigone. The walls were thickened and trabeculated. A fibrous stricture was present in the membranous urethra. Distributed through the entire colon there were numerous circumscribed, reddish gray ulcers averaging 1 cm. in diameter. Qualitative tests for mercury on portions of the kidney and colonic contents were positive.

Histological Examination: Extensive bronchopneumonia, acute purulent bronchitis, and cloudy swelling of the myocardium were present. There was diffuse and uniform fine granularity and swelling of the liver cells throughout the lobules. Some of the liver cells showed dark brown pigmentation and the sinusoids were moderately engorged with red blood cells. The nuclei of the liver cells were irregular in size, vesicular, and varied greatly in their chromatin content. No evidence of necrosis or regeneration could be seen. In the colon there was edema, necrosis and desquamation of the mucosal epithelium. The gland spaces were represented by an acidophilic debris, with cell outline not discernible. The nuclei appeared fairly intact in the places where the epithelium was not desquamated. There was a moderate degree of polymorphonuclear cell infiltration of the mucosa. The submucosa was edematous and hyperemic.

Examination of the kidney revealed degeneration and necrosis in all parts of the tubular system except the glomerular capsules. This was most severe in the convoluted tubules, both proximal and distal, with slightly less involvement of the loops of Henle and collecting tubules. Corresponding anatomical portions of different tubules in the same kidney varied in the degree of their damage.

There were two types of epithelial cell necrosis. The first was a coagulation necrosis characterized by small, sharply outlined cells having a homogeneous, basophilic cytoplasm. The nuclei were pyknotic or absent. This type occurred almost exclusively in the convoluted tubules. The second type was characterized by large, acidophilic, granular swollen cells which in the more advanced stages of their destruction had indefinite outlines, with ragged inner borders

and large, vesicular or fragmented nuclei, (Fig. 1). This type of necrosis predominated and occurred in all tubules. Not all the necrotic epithelium had desquamated, although in many of the tubules the basement membrane was entirely denuded. Many of the tubular luminae contained coagulated, swollen, disintegrating necrotic epithelial cells. Others contained apparently living cells with clear, distinct, non-granular cytoplasm and intact nuclei. Tubules lined with epithelium showing the second type of necrosis often contained acidophilic vesicular, granular debris. This was canalized and formed bridges connecting the adherent layers of disintegrating cells. There were occasional acidophilic hyaline as well as granular casts. Many of the tubules were filled with red blood cells, and many others with polymorphonuclear leukocytes.

Calcification occurred only in tubules the seat of the first, or coagulation type of necrosis, and was of only slight degree. Occasional calcified or partially calcified cells were seen in the lumina; others were still attached to thin basement membranes.

Regenerated epithelium appeared as low flattened cells with clear cytoplasm and distinct outlines. The nuclei were intact and hyperchromatic. In tubules where regeneration was slight, probably early, the nuclei appeared elongated. Occasional small mitotic figures were observed. Some of the tubules were completely lined with regenerated cells, and others showed beginning regeneration beneath the necrotic epithelium, so that there were two or three layers of cells. Some tubules were so completely filled with regenerated cells that the lumen was occluded.

Many of the glomeruli were large, hypertrophic and cellular, while others were atrophic and hyalinized. The capsules were thickened, crescentic, and many were adherent to the glomerular tufts.

The interstitial tissue was edematous, and showed areas of diffuse polymorphonuclear cell infiltration, as well as localized abscesses containing the same type of cells. There was some interstitial hemorrhage. No relationship between the infiltrating cells and the vascular supply was observed.

Many of the small blood vessels were thick-walled, and some were completely obliterated by a proliferation of the intimal layer. The large vessels showed no changes.

The diagnosis was mercury poisoning, acute nephrosis and pyelonephritis superimposed on chronic glomerulonephritis and arteriolar

nephrosclerosis; confluent bronchopneumonia of both lower lobes; parenchymatous degeneration of the liver; hemorrhagic colitis; cystitis; stricture of the membranous urethra.

CASE II. Clinical History: E. S., a white female 35 years of age, was admitted to the Cleveland City Hospital on October 18, 1927. The patient's complaints were sore mouth, vomiting, and inability to urinate. In June the patient went to the "blood serum clinic" in question, and was told she had a four plus Wassermann, for which she took a course of treatments at the "clinic," and had received a total of twenty-five injections during the summer and autumn. The last one was received five days before admission. On the day preceding her last injection she had noticed that her urine was red and within an hour after receiving her last injection there was dizziness, nausea and dryness of the mouth. This was soon followed by much vomiting. Prior to her admission to the hospital she had a few small bowel movements which did not appear bloody. She passed no urine for five days prior to her admission on October 18, and none was obtained by catheterization. She had a history of a secondary eruption seven years ago, and had been treated with injections and mercury rubs. There had been numerous miscarriages since that time, all of them being at about two months.

Examination revealed a rational patient, vomiting considerably. The mouth was sore, lips were dry, and tongue was coated, with many small ulcers on the under surface. The teeth were loose, there was a marked gingivitis and the submaxillary glands were swollen and tender. The abdomen was distended and tympanitic, but no tenderness, or masses were revealed. The remainder of the examination was negative. Respirations were 20, pulse 80 to 90 and blood pressure systolic 132 and diastolic 84.

Laboratory Findings: No specimen of urine was obtained until the fourth hospital day. The first one was small in amount and contained gross blood. The second was clear, alkaline, and had a specific gravity of 1.008. It contained a small amount of albumen and sugar and numerous red blood cells and leukocytes but no casts. On the following day it was cloudy, alkaline, with a specific gravity of 1.012 and gave a positive test for albumen but was negative for sugar. Microscopic examination showed hyaline casts, many red blood cells and leukocytes. On admission she had a leukocyte count of 13,300, red blood cell count of 3,990,000 and 75 per cent hemoglobin. Chemical analysis of the blood on October 20 showed urea 237 mg. per 100 cc., creatinine 6.3 mg. per 100 cc., uric acid 5.3 mg. per 100 cc., chlorides 412 mg. per 100 cc.

The patient received sodium thiosulphate intravenously, saline infusions, and high colonic irrigations. Her course was a febrile throughout. She died at 10:45 P.M. on October 24, six days after admission and eleven days after her last injection.

Autopsy: Performed at the morgue by the county coroner, Dr. Hammond, and to him I owe my thanks for permission to use his findings. The right arm and forearm were swollen and discolored on the volar surface, about the region of the median basilic vein. The heart was negative, but the lungs showed a moderate amount of edema and congestion. The report stated that there was fatty

degeneration throughout the liver. The kidneys were large and together weighed 470 gm. They were pale both externally and on section. The glomerular markings were visible, and there were radial striae in the cortex. The boundary between cortex and medulla was indistinct. The spleen was large, weighing 180 gm. It was dark red in color, soft in consistency, and the follicular markings were not obscured.

A quantitative determination for mercury showed none in 100 cc. of blood, none in a portion of kidney, and 3.5 mg. in a 15 gm. section removed from the wall of the large bowel.

Histological Examination: There were no abnormalities of the myocardium and the lungs showed a slight amount of congestion and edema. The sinusoids of the spleen were engorged with blood. The liver cells were moderately swollen and granular, all portions of the lobule being uniformly involved.

Sections of the colonic wall showed no change other than what appeared to be autolysis of the mucosa.

Sections of the kidneys showed diffuse degeneration of the epithelium in all portions of the tubular system except the glomerular capsules. The tubules were distended, and the loops of Henle and collecting tubules showed more damage than did those in the preceding case.

The first type of necrosis, the coagulation type, predominated, and was as severe in the loops of Henle as in the convoluted tubules. Necrotic cells with swollen, acidophilic, granular cytoplasm and vesicular nuclei were seen but were not conspicuous. In many tubules desquamation was complete, leaving a denuded basement membrane. Others were partially or completely lined by necrotic epithelium.

Casts were few and consisted predominantly of epithelial cells which had undergone coagulation necrosis. Hyaline casts were seen. Some of the most widely dilated tubules were filled with an acidophilic, granular, vesicular substance.

There was considerable calcification of the necrotic epithelium. This was observed in that which was still attached to the tubular wall, as well as in masses of completely desquamated cells. It occurred only in those cells showing the coagulation type of necrosis.

Regenerated epithelial cells were present but not abundant. Where they occurred, they undermined necrotic cells, but the tubules were not as completely filled as in the preceding case. The regener-

ated cells presented the same details of structure as previously described.

There was much edema, as well as a moderate degree of lymphocytic infiltration of the interstitial tissue, (Fig. 4).

The glomeruli and blood vessels showed no changes.

The diagnosis was acute nephrosis; parenchymatous degeneration of the liver; pulmonary edema; passive hypermia of the lungs and spleen.

CASE III. Clinical History:* J. K., a white male, aged 49 years, was admitted to the Cleveland Clinic Hospital on October 21, 1927, with a history of anuria for one week. The patient had a sudden chill eight days previously while riding home on the street car from a visit to the "blood serum clinic," where he had received an intravenous injection. On reaching home his temperature was 102° F. There was severe nausea, emesis, and pain in the abdomen. The following day he had diarrhea with bloody and mucous stools, and stopped passing urine. After three days of these manifestations he felt better for a day and then became worse. By this time a marked stomatitis had appeared. Nausea and vomiting persisted. The stools were frequent (as often as every hour on some days) and continued to be bloody.

On admission his temperature was 36.8° C, pulse 86, and blood pressure systolic 130 and diastolic 70. Examination showed a well developed and nourished male with marked herpes about the lips, stomatitis, foul, coated tongue and uriferous breath. There was tenderness over the entire abdomen, with a slight degree of spasticity. There was bleeding from the rectum.

Laboratory Findings: The blood showed 4,120,000 red blood cells, 85 per cent hemoglobin, and 17,300 leukocytes. A differential count revealed 90 per cent polymorphonuclear leukocytes and 10 per cent lymphocytes. There were 312 mg. of urea, 182 mg. of sugar, 6.6 mg. of uric acid and 465 mg. of chlorides per 100 cc. of blood. The blood Wassermann was four plus and the Kahn test three plus. The stools gave a strongly positive benzidine test, and bright red blood was visible grossly.

Treatment consisted of saline infusions and the intravenous injection of sodium thiosulphate. The patient became irrational on the morning after admission, lapsed into coma, and died in the afternoon. His temperature remained below normal until just before death when it rose to 36.8° C. His pulse varied from 90 to 100 and his respirations from 20 to 25. Anuria persisted during his stay in the hospital. At no time did he have convulsions.

Autopsy: The autopsy was performed four hours after death. The gums showed a bluish tinge. There was pitting of the chest wall, but no edema of the extremities. The colon was covered with glistening peritoneum. There was no free fluid in any of the serous cavities. The lungs showed a moderate degree of congestion and

* I wish here to acknowledge my sincere thanks to Dr. Phillips and Dr. Ball for permission to report this case.

edema. The heart, spleen, stomach, duodenum and jejunum showed no gross changes. Beginning about one meter proximal to the ileocecal valve there was a reddened and congested appearance of the mucosa. There were occasional superficial ulcerations averaging 1 to 2 mm. in diameter in the free margins of the mucosal folds. Such areas were hemorrhagic, with small blood clots attached to the ulcers. They were irregular in distribution. The mucosa of the cecum and colon throughout its extent showed irregularly shaped and distributed superficial ulcerations averaging 8 by 3 mm. and having attached blood clots. The intervening mucosa was dark red. The colon was filled with a bright red, thick fluid.

The liver weighed 1450 gm., was smooth, dark brown in color, and had slightly rounded margins. The cut surface showed a normal architecture.

The left kidney weighed 170 gm., the right, 165 gm. They were firm, smooth, and showed a yellowish gray cut surface. The cortex was 7 mm. thick and well differentiated with pale yellowish radial markings.

Pelves, ureters, bladder, prostate and adrenals all grossly appeared normal.

Small portions of the liver (20 gm.), colon (10 gm.) and kidney (10 gm.) gave strongly positive qualitative tests for mercury. Electrolytic quantitative tests showed 10 mg. of mercury in 15.5 gm. of colonic contents.

Histological Examination: There was slight cloudy swelling of the heart muscle cells. The lungs showed a very early bronchopneumonia and considerable congestion and edema. Passive hyperemia of the spleen was found. The gastric mucosa showed no change other than autolysis, and the ileum was unchanged. There was coagulation necrosis of the colon mucosa, with diffuse and focal areas of polymorphonuclear leukocytic infiltration of the mucosa and submucosa. In addition, the submucosa showed much congestion and hemorrhage. The liver cell cytoplasm was diffusely swollen and granular. The blood vessels were filled with red blood cells but the sinusoids were empty. There was swelling, vacuolization and granularity of the adrenal cells, diffuse in the medulla, but only occasional cortical cells were involved.

The tubular damage in the kidneys of this case was less extensive and severe. Only the convoluted tubule epithelium showed necrosis,

while the collecting tubules and loops of Henle were the seat of moderately severe parenchymatous degeneration.

Both types of necrosis were observed, with a slight predominance of the coagulation variety. Much of the necrotic epithelium was desquamated, and some detached epithelium which appeared to be living was observed. In the loops of Henle and collecting tubules the cytoplasm was swollen, finely granular and showed some fat vacuolization. The nuclei were intact. There was little desquamation of the epithelium in these tubules.

All tubules contained many hyaline casts in addition to masses of desquamated epithelium. No red or white blood cells were seen in the lumina.

There was much calcification of the necrotic cells. It was seen in desquamated epithelium as well as in that which was still attached (Fig. 3).

Regenerated epithelial cells occurred as a thin layer of flattened epithelium on the basement membrane, with intact nuclei and cytoplasm. Occasional mitotic figures were seen. A few tubules were found where the regenerated layer of cells was overlaid with necrotic, incompletely desquamated cells.

Interstitial edema was present but not so pronounced as in the two preceding cases. A few focal areas of lymphocytic infiltration of the interstitial tissue were present in the cortex.

Except for an occasional hyalinized glomerulus, the glomeruli showed no changes.

The medullary vessels were engorged with red blood cells, but there were no changes in the vessel walls.

The diagnosis was acute nephrosis; hemorrhagic colitis; early bronchopneumonia; parenchymatous degeneration of the liver; pulmonary edema and congestion; cloudy swelling of the myocardium.

CASE IV. Clinical History:* C. V. B., a white male 60 years of age was admitted to Lakeside Hospital on October 22. The patient gave a history of having had three intravenous injections within five days. These were to constitute a "course" to cure "neuritis" in his left shoulder and to lower his blood pressure. The patient was not a luetic. After having his second injection he thought he did not feel as well as before, and after his third injection he became dizzy and weak. He was confined to his bed and finally referred to the hospital by his private physician. There had been no anuria, hematuria or bloody stools.

On admission the patient was rational and talkative. There was blood-

* I am indebted to Dr. M. A. Blankenhorn for permission to report this case.

stained sputum in his mouth, and his tongue and mucous membranes were dry. The gums were swollen, soft, spongy, dark, and showed minute hemorrhagic spots. The abdomen was distended and tympanitic. There was no edema.

After two days his condition seemed improved. He had been passing fairly copious quantities of urine of low specific gravity (1.010) which did not vary. There was a moderate amount of albumen. Pus cells were numerous and a few hyaline casts were seen. The leukocyte count was 12,000. The red cells and hemoglobin were within normal limits. His blood pressure was systolic 180 and diastolic 90. Phenolsulphonephthalein tests were done on successive days (fourth and fifth) with no excretion of the dye in two hours. On the fourth day his blood urea was 187 mg. Toward the latter part of his stay in the hospital he had incontinence of urine and feces. His stools were tarry, and later stained with fresh blood. Throughout his stay his respirations were slow, and his temperature was slightly elevated until the last day, when it became subnormal. On the last day at 10 A.M. he developed sudden pulmonary edema, foamy material coming from his mouth and nostrils. This cleared up after 1/100 gr. of atropine was given hypodermically, but the patient died eight hours later, on the eighth hospital day.

Autopsy: The postmortem examination was performed about three hours after death. No edema was observed. The neck veins were engorged. The gums were swollen, soft and spongy, showing a few small hemorrhagic areas. The buccal mucosa was hyperemic. No free fluid was encountered in any of the serous cavities. The stomach and colon were dilated. There was some catarrhal exudate in the lumen of the trachea and bronchi. The heart showed no abnormalities. A few small yellowish plaques of intimal thickening were encountered in the arch and ascending portions of the aorta. The liver weighed 1575 gm. and was fairly firm in consistency. It was pale, and on cut section the architecture was somewhat obscured. The spleen weighed 180 gm., was firm in consistency, and showed considerable passive hyperemia. The kidneys weighed together 500 gm. The capsule stripped readily, leaving a pale red, smooth surface. The cortex was pale and slightly thickened but showed radial striations. The pyramids were engorged with blood, and presented a marked contrast to the pale, swollen appearance of the cortex. The bladder was thick-walled and trabeculated. The prostate showed a diffuse nodular enlargement. The esophagus, stomach, and small intestines showed no changes. Beginning at the cecum and extending throughout the length of the colon, the mucosa was hyperemic, edematous, and showed minute hemorrhagic areas with no ulceration or necrosis. The brain showed no changes but there was edema of the leptomeninges.

A quantitative determination gave 11.09 mg. of mercury in one entire kidney.

Histological Examination: Sections of the lungs showed some of the bronchi to be partially and others completely filled with an exudate containing polymorphonuclear leukocytes. The bronchial walls showed no change, however, and the alveolar walls and septa appeared normal. In the liver there was much fatty vacuolization of the cells in the central areas of the lobule, extending about halfway to the peripheral zone. There was granularity, vacuolization, and variation in chromatin content of the nuclei. In the peripheral zones the cells were swollen and granular, but the nuclei appeared undamaged, and there was less vacuolization of the cytoplasm. The splenic vessels and sinusoids were engorged with blood. Sections of the colon showed necrotic epithelium of the mucosa with a hemorrhagic stroma and edematous submucosa. The medullary portions of the adrenal showed swollen and vacuolated cells with granular cytoplasm. The cortical cells appeared unchanged. The prostate was hypertrophic.

In this case the tubular epithelial cell degeneration and necrosis in the kidneys were less pronounced than in any of the others. The convoluted tubules were the most severely damaged, the collecting tubules showing no damage other than moderate cloudy swelling. In the loops of Henle there was a degree of damage intermediate between that of the convoluted tubules and the collecting tubules.

Necrosis of both types of about equal extent was seen, but there was relatively little desquamation.

Masses of necrotic, desquamated epithelial cells, hyaline casts, red blood cells, and white blood cells were all seen within the lumina of the tubules. Calcification was not present.

Regeneration of the tubular epithelium was the prominent feature. Some tubules were lined with flat, newly formed epithelium similar in appearance to that in the preceding cases. Part of these were overlaid with irregular layers of necrotic epithelium. Others were completely lined with a single layer of the newly formed cells. A conspicuous feature, however, was the occurrence of newly formed epithelial cells, rich in cytoplasm, having hyperchromatic nuclei, and frequently showing a piling up of the cells two and three layers deep. Mitoses were frequent, (Fig. 2).

The glomeruli showed no changes.

In the interstitial tissue there was a moderate degree of edema. Patchy areas of lymphocytic and polymorphonuclear cell infiltration were seen in the regions of tubules containing white blood cells. There were no vascular changes.

The diagnosis was mercury poisoning; acute nephrosis; hemorrhagic colitis; parenchymatous degeneration of the liver; pulmonary edema; congestion of the lungs and spleen; hypertrophy of the prostate.

DISCUSSION

Degeneration and necrosis were observed in the tubular epithelium of all four of these cases. The epithelial damage ranged from parenchymatous degeneration to necrosis with calcification. The most severe lesions were seen in the proximal and distal convoluted tubules, while the loops of Henle and the collecting tubules were involved to a lesser degree. In Cases I and II there was marked degeneration and necrosis present in all parts of the renal tubular system, except for the glomerular capsule, while in Cases III and IV the injury to the loops of Henle and the collecting tubules was manifested only by parenchymatous degeneration. In only one of the cases (Case III) was there any considerable fatty degeneration of the tubular epithelium.

Two types of cell death were seen. Simple coagulation necrosis of epithelium was the more common, the cell outline being left intact with pyknosis or disappearance of the nucleus. Such cells were seen still attached to the basement membrane as well as free in the lumen. Frequently, however, the cell death was manifested by a marked swelling, the inner margin being ragged and the nucleus large, vesicular, and fragmented. In tubules whose epithelium was the seat of this type of necrosis, the lumen was often filled with a vesicular acidophilic granular material resembling the degenerated cytoplasm which occasionally bridged the tubule from one side to the other. These changes were not constant in their location even in the same kidney but all the kidneys examined showed a co-existence of both types of necrosis.

The presence together of both types of cell death is of interest in connection with MacNider's ² findings in a study of the renal injury of dogs following mercuric chloride poisoning. He concluded that the primary nephrotoxic action of mercury is predominantly on the tubular epithelium and consists of diffuse coagulation necrosis with-

out edema, and that the swelling and necrosis of epithelium with fragmentation of nuclei are associated with the acid intoxication and anuria which appear later. The latter type of necrosis developed as late as the ninth day of intoxication when the urine no longer contained mercury. The presence of diffuse coagulation necrosis in the kidneys of our cases as late as the twelfth day of the intoxication indicative, according to MacNider, of the nephrotoxic action of mercury would imply a very delayed excretion of the poison. This indicates that a large part of the intravenous doses of mercury was fixed and slowly liberated.

Tubular casts of many kinds were seen. Pus cell casts were present in Cases I and IV, while red blood cells were present in the tubules of Cases I, II and IV. Cellular casts of coagulated necrotic epithelium as well as disintegrated swollen necrotic cells were seen in all four cases. In addition there were in all cases, rounded, apparently living epithelial cells with intact, normal appearing nuclei free in the tubules. Hyaline and granular casts were present in all cases, the latter being often vesicular and associated with necrosis in the lining epithelium.

Calcification of necrotic epithelium was present in the first three cases and was more marked in Case III. The kidneys of this case likewise showed the most extensive coagulation necrosis of tubular epithelium. No calcification of apparently living cells was seen and no evidence was found to support Leutert's ⁵ contention that calcium deposition occurs in injured but still functioning cells. The necrotic cells showing calcium deposition occurred in masses free in the lumen of the tubules as well as in their original places in the lining of the tubules. These calcified cells were more abundant in the cortex but were occasionally seen in Henle's loops or even the collecting tubules.

Regeneration of the tubular epithelium was seen in all four cases and was most extensive in Case IV in which tubular damage and calcification were least manifest. As described by Heineke ¹ the earliest stage of regeneration consisted in the formation of a flattened layer of young cells between the necrotic epithelium and the basement membrane of the tubules. He ascribed the desquamation of the degenerated and necrotic cells to this proliferation but we observed occasional tubules completely denuded of their epithelium with no evidence of epithelial regeneration. In Case IV the epithelial regeneration was quite exuberant, many of the tubules being almost

occluded by the young cells with a large amount of cytoplasm and nuclei of varying size and chromatin content. Small mitotic figures were observed and in places the new epithelium was piled up several layers deep. The contrast between the young and the swollen degenerated old epithelium was sharp because of the definite cellular outline, the acidophilic, clear cytoplasm and the absence of vesicular and fragmented nuclei in the regenerated epithelium. No evidence of new tubule formation was observed. The most pronounced epithelial regeneration occurred in the convoluted tubules which were likewise the seat of the most severe degeneration. The glomeruli were hyperemic but there was no other change except for the chronic diffuse glomerulonephritis seen in Case I and the scattered hyalinized glomeruli seen in Case III.

The interstitial tissue was edematous in the four cases with patchy lymphocytic infiltration. Case I was complicated by pyelonephritis and showed abscesses throughout the cortex with extensive peripheral zones of leukocytic infiltration. In Case IV there was interstitial leukocytic infiltration around some of the pus-containing tubules. Except for the complicating arteriosclerosis in Case I there was no evidence of vascular change. Neither the thrombosis with multiple infarction observed by Kaufmann⁶ nor the perivascular infiltration reported by Karvonen⁷ occurred in these cases.

Damage to the liver has been noted by many investigators of the pathological changes incident to mercury poisoning. Burmeister and McNally⁸ considered the degeneration in the liver to be due to the action of mercury as such during absorption and elimination. They found the liver injury to vary with the duration of the intoxication and the kidney injury to vary with the amount of mercury introduced as well as with the duration of the intoxication. They observed a marked parenchymatous degeneration of the hepatic cells of the central zones. MacNider^{2,9} in experimental studies on animals in which the mercury was introduced by stomach tube reported the occurrence of edema and necrosis in the periphery of the liver lobules. Foster¹⁰ called attention to the degeneration of liver cells in a fatal case of mercurial poisoning and Turrettini and Piotrowski¹¹ described two cases of fatal mercuric poisoning with liver damage so severe that they considered the liver injury to be the cause of death.

We observed a uniformly mild grade of parenchymatous degenera-

tion of the liver in all of our cases with no necrosis and no selective localization to any part of the lobules. This raises the question of whether the severe hepatic damage is due to the enteral route of intoxication and therefore not present in intravenous poisoning.

Epithelial necrosis and ulceration in the colon was a constant finding and all of the cases showed lesions of varying severity in the large bowel. Cases II and IV showed simple epithelial necrosis while the entire colon of Cases I and III was the seat of multiple deep hemorrhagic mucosal ulcers. There was no gastro-enteritis in any of the cases.

SUMMARY AND CONCLUSIONS

1. The four cases here reported survived periods of intoxication ranging from six to twelve days following the intravenous injection of mercuric chloride in a dosage of over 5 mg. per kilogram of body weight.

2. Two types of necrosis were found in the kidneys of each of the four cases.

3. The desquamation of renal tubular epithelium was not necessarily dependent upon epithelial regeneration beneath the necrotic cells.

4. Calcification was observed only in epithelium the seat of coagulation necrosis and the calcified cells were seen *in situ* as well as free in the lumina of the tubules.

5. The liver damage in these cases consisted only of a mild parenchymatous degeneration in contrast to the severe hepatic changes observed after mercury poisoning by mouth.

6. Epithelial necrosis and mucosal ulceration of the colon were noted in the four cases.

7. The renal changes due to mercury poisoning in man are essentially the same whether the mercury is administered by mouth or by the intravenous route.

8. Gastro-enteritis does not occur after the intravenous injection of large doses of mercury.

I wish to record my sincere thanks to Dr. Alan R. Moritz, the pathologist in charge at Lakeside Hospital, for his kind assistance and advice in the preparation of this report.

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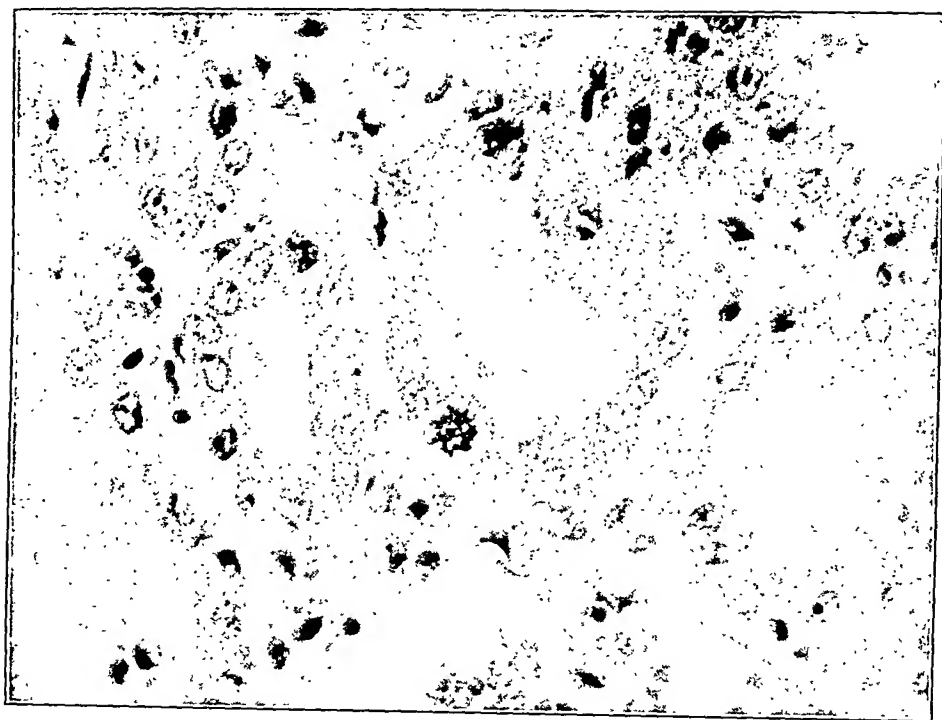
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 DESCRIPTION OF PLATES

PLATE 76

FIG. 1. Case I. Hematoxylin and eosin stain, showing swollen type of epithelium necrosis and regeneration, with mitotic figure. $\times 475$.

FIG. 2. Case IV. Showing coagulation necrosis of tubular epithelium and piling up of regenerating cells in one of the tubules. $\times 250$.



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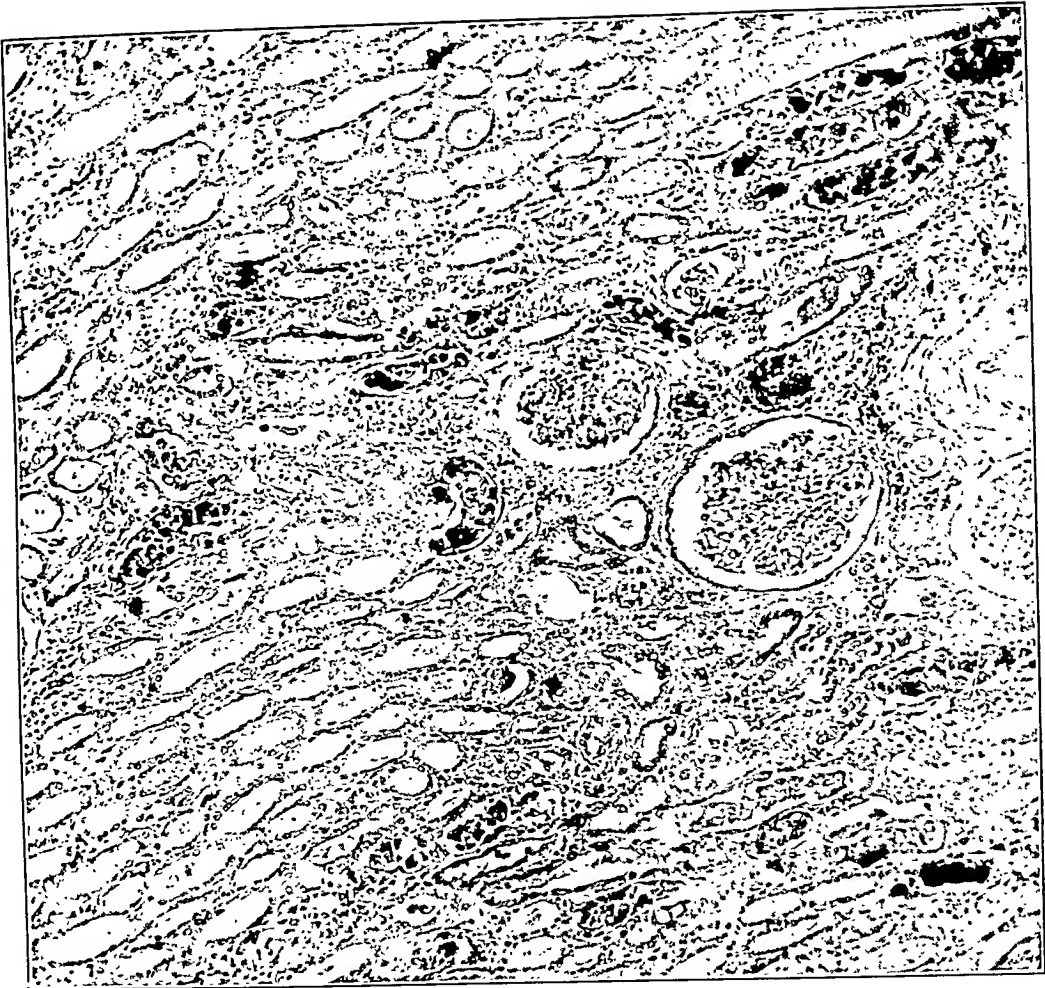


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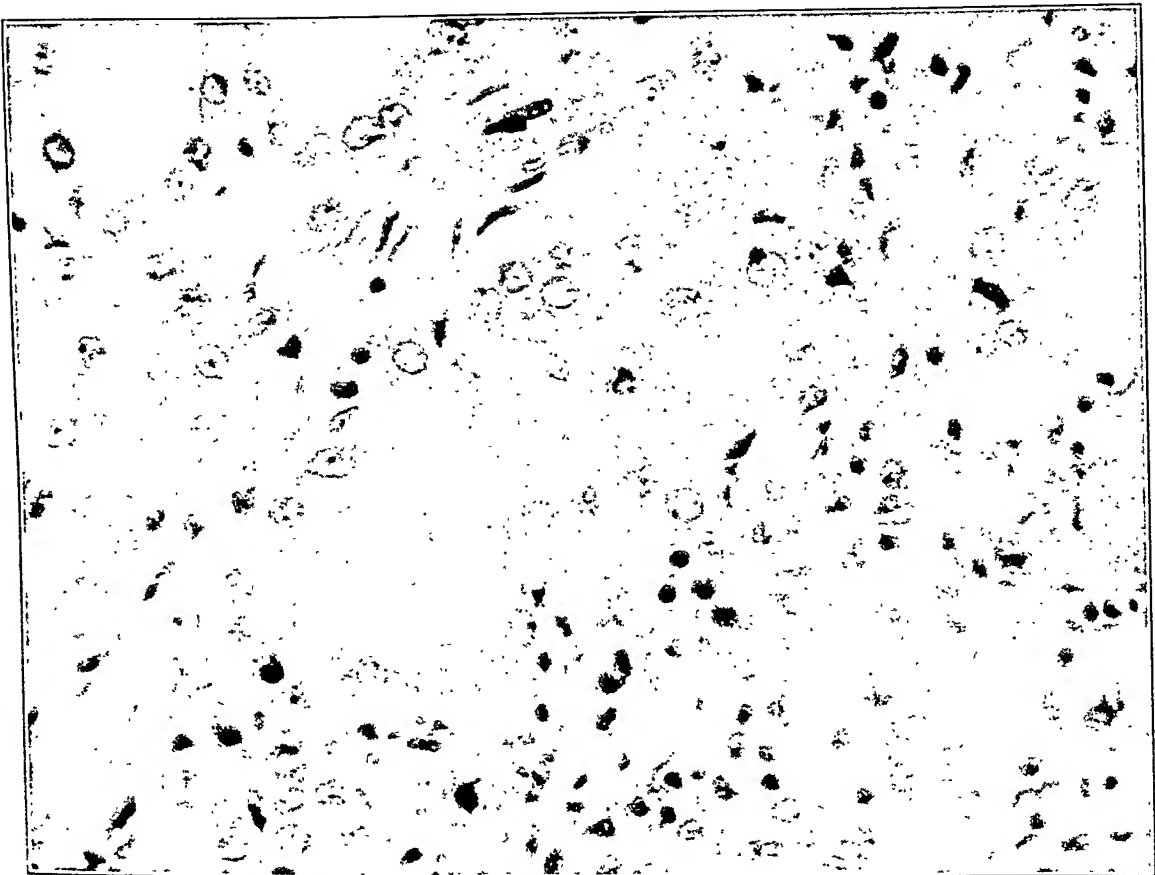
PLATE 77

FIG. 3. Case III. Low power magnification showing coagulation necrosis and calcification of necrotic epithelium.

FIG. 4. Case II. Showing interstitial edema and lymphocytic infiltration. Tubules show swollen type of epithelial necrosis and bridging of lumen by necrotic cytoplasm.



3



4

TISSUE CULTURE OF INTRACRANIAL TUMORS

WITH A NOTE ON THE MENINGIOMAS

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It has been the purpose of this clinic for some time to utilize the tissue culture technique in the study of intracranial tumors. It was thought that observations on living tumor cells cultivated *in vitro* might profitably supplement the usual histological examination of fixed tissue and add not a little to our present knowledge of their histogenesis. This present paper records the results of some preliminary studies in this direction.

TECHNIQUE

The technique employed is the hanging-drop method of Lewis and Lewis.¹ A bit of healthy tumor tissue secured at operation is cut up in Locke or Tyrode solution into small pieces a millimeter or less in diameter. A piece is placed in the center of a clean sterile coverslip and a drop of heparinized human plasma is added. The coverslip is then inverted and sealed by means of a vaseline ring on a depression slide. In some cases a drop of tumor extract is added to promote growth. Aseptic precautions are observed throughout. The cultures are incubated at 37.5° C.

In successful cultures (Fig. 1) the cells migrate out within twenty-four hours on the under surface of the coverslip. One can then study under the highest powers of the microscope the morphology of the living cells as well as their reaction to vital dyes and to particulate matter.

TUMORS CULTURED

In order to ascertain what types of tumors would be favorable material for cultivation all tumors removed on the neurological service over a period of several weeks were cultured. A list of these

* Received for publication May 12, 1928.

together with the percentages of success with each is shown in the table below:

TABLE I
Tumors Cultured

	No. cultured	Satisfactory	Per cent
Meningioma.....	5	3	60
Metastatic carcinoma.....	2	1	50
Spongioblastoma multiforme.....	1	1	100
Pituitary adenoma.....	5	0	0
Astrocytoma.....	3	0	0
Acoustic neuroma.....	2	0	0
Cystic spinal tumor.....	2	0	0
Cysts.....	3	0	0
Unclassified malignant epithelial tumor.....	1	0	0
	<hr/>	<hr/>	<hr/>
Total	24	5	21

The results were not satisfactory in many of the tumors. With the simple technique employed one could hardly expect the cells of slowly growing tumors like the astrocytomas to migrate actively in tissue culture. In a few of the pituitary adenomas a small number of epithelial cells wandered out from the explanted piece, forming a loose sheet; a growth pattern characteristic of glandular epithelium. In cultures of two cystic tumors of the spinal cord ciliated epithelial cells migrated out.

Satisfactory growth occurred in cultures of tumors of three types: meningioma, metastatic carcinoma, and spongioblastoma multiforme.* The success with the one specimen of spongioblastoma inspires the hope that the gliomas of relatively undifferentiated cell type may prove to be favorable material for study by tissue culture methods.

MENINGIOMAS

Active migration occurred in cultures of three out of five meningiomas. The cultures were kept alive for as long as three weeks by renewing the medium every three or four days and, in a few cases by subculturing the explanted piece. Attempts were made in our observations on these cultures to determine the precise nature of the outwandering cells.

The typical cells that grew out seemed to be of the mononuclear-

* Since Mr. Kredel made this preliminary study in the summer of 1927 we have had success with other types, notably with the acoustic neurinomas. H. C.

macrophage series rather than fibroblasts. Most of the cells were ameboid in shape. While some of them were pyriform and unipolar, the multipolar stellate form with many cell processes characteristic of fibroblasts was not in evidence during the first few days after explantation. Some cells showed a rosette of neutral red granules in the region of the centrosphere. In older cultures, however, the cells appeared gradually to assume a form more closely resembling that of fibroblasts.

To check these morphological observations with some physiological criterion the phagocytic power of the outwandering cells was tested by adding to the cultures a suspension of finely divided carmine in Locke solution. In cultures of a few days the cells ingested large numbers of carmine particles. Fig. 2 is from a seven-day culture of meningioma. This photograph illustrates both the characteristic morphology and the remarkable phagocytosis of carmine noted in the younger cultures.

After cultivation for a week or more the cells seemed to lose their phagocytic ability. Fig. 3 shows a group of cells in an eleven-day culture from the same tumor as the cells shown in Fig. 2. These latter cells did not phagocytize carmine to any noticeable extent. An anomalous result was obtained with a twenty-day culture, a field of which is shown in Fig. 4. The cells failed to ingest any carmine during the first half hour after the suspension was added. But at the end of four hours the cytoplasm had become tremendously swollen, distorted, and contained many carmine particles. We are inclined to believe that this last was an artifact due to the basicity of a poorly prepared carmine suspension, the reaction of which was found to be pH 7.9.

These observations, although suggestive, do not add much of critical importance to our present knowledge of the histogenesis of the meningiomas. If the view of Mallory² and Penfield³ is correct that the type cell of these tumors is the fibroblast, the outwandering cells in our cultures must be clasmatocytes present in the stroma. This possibility must be considered, for Lewis and Gey⁴ have shown that clasmatocytes migrate out abundantly in cultures of mouse sarcoma and have pointed out that the presence of large numbers of such phagocytes may well be overlooked in fixed sections. On the other hand, these observations conflict in no way with the view of Cushing^{5,6} that the histogenesis of the meningiomas is explained

on the basis of tumefaction of clusters of meningocytes that line the arachnoid villi and which possess phagocytic powers. The meningiomas when examined fresh by supravital technique are found to contain large numbers of these phagocytic cells and in his opinion they presumably represent a constituent part of the tumor.

SUMMARY

Successful tissue cultures with the hanging-drop technique have been made from meningioma, metastatic carcinoma, and spongioblastoma multiforme.

Phagocytic cells migrated out in large numbers in meningioma cultures. The ability of these cells to ingest particulate carmine decreased progressively with the age of the cultures.

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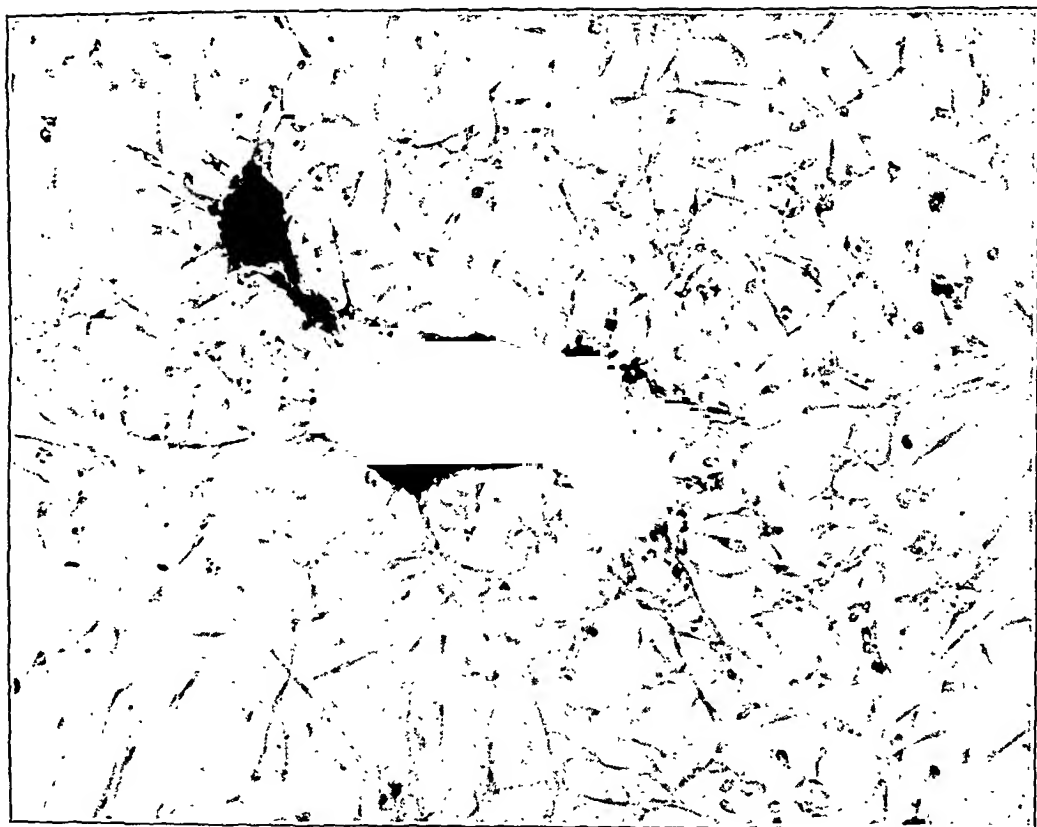
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DESCRIPTION OF PLATES

PLATE 78

FIG. 1. An eight-day culture of a metastatic carcinoma (fixed in formalin, H. and E stain), showing an abundant outgrowth of multipolar and often multinuclear cells containing large granules not taking neutral red. $\times 100$.

FIG. 2. A typical field of growing meningioma (seven-day culture, H and E stain). Most of the cells have ingested large numbers of carmine particles and are obviously phagocytic. $\times 600$.



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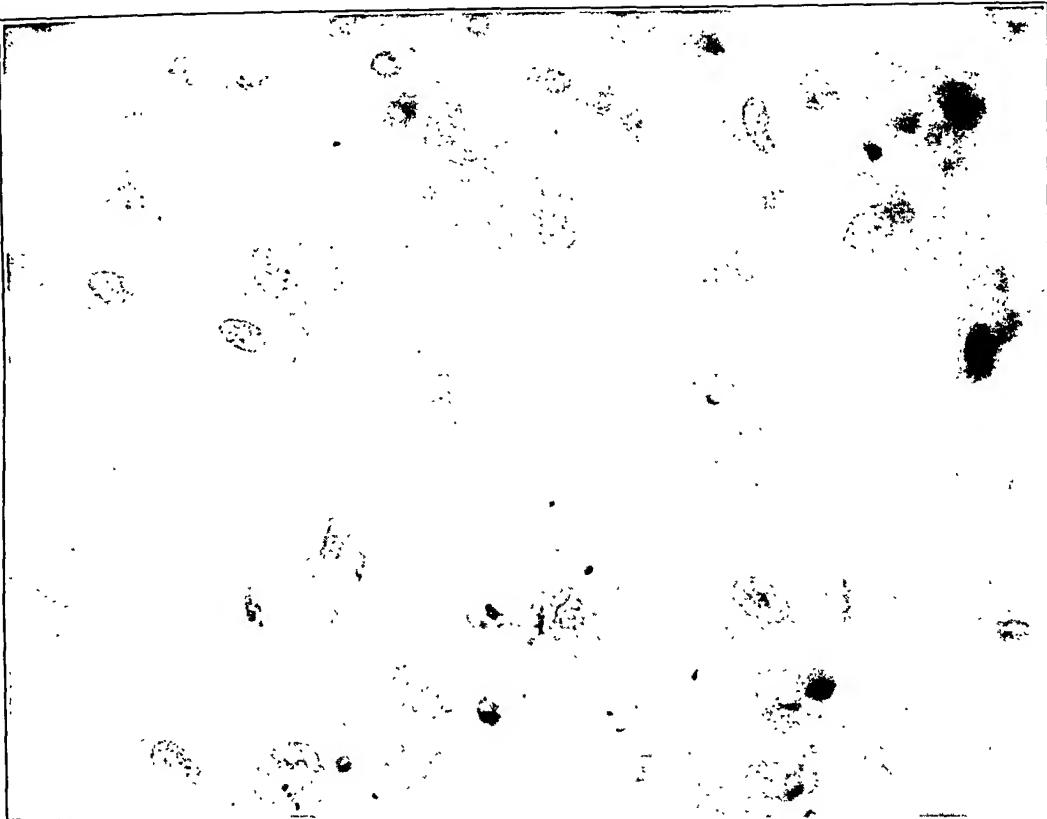


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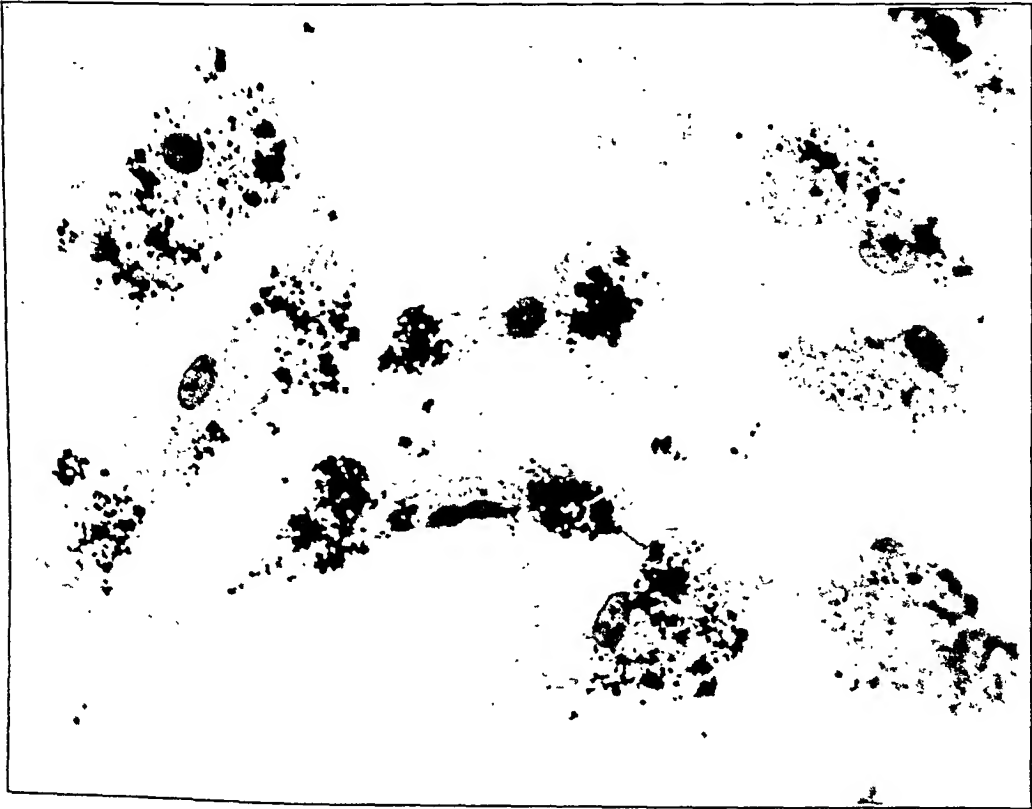
PLATE 79

FIG. 3. An eleven-day culture of same tumor as Fig. 2 (H and E stain), showing cells (fibroblasts or meningocytes?) which do not ingest carmine. $\times 600$.

FIG. 4. Cells from same tumor as Fig. 2 after twenty-day growth (H and E stain), showing them richly laden with carmine particles. $\times 600$.



3



4

A STUDY OF THE TISSUE CHANGES IN EXPERIMENTAL BLACK TONGUE OF DOGS COMPARED WITH SIMILAR CHANGES IN PELLAGRA*

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INTRODUCTION

In the present communication there is presented a report on the gross and microscopic changes in a graded series of early and late cases of experimental black tongue of dogs. This study was made in connection with, but essentially independently of, the study of experimental black tongue being conducted at the Hygienic Laboratory by Goldberger, Wheeler, Lillie and Rogers.¹ The material was secured from dogs that had served as test animals in certain of the feeding experiments carried out by Goldberger and associates in the course of their study of black tongue. These workers furnished the summary statement, presented in the appendix, of the significant points in the history of each of the animals from which tissues were secured.

Technic: The animals were killed with illuminating gas and careful autopsies were done immediately after death. Tissues for histologic study were fixed in a modified Zenker's fluid (that is, Zenker's without acetic acid) and stained with Giemsa's stain. Formol fixation and hematoxylin-eosin staining were also used in some cases. Weigert preparations were made on sections of the spinal cord in the later cases of the series. Every important organ and tissue was examined histologically in each case.

GROSS PATHOLOGY

Care was taken not to confuse the lesions of the disease under consideration with the effects of partial starvation which at times complicated it. The most typical and important lesions appeared in dogs still in good states of nutrition. The effects of partial starvation were seen in three animals killed late in the series. They will

* Received for publication May 15, 1928.

not be described as they are not pertinent to the condition under consideration.

Mouth: The earliest recognizable lesion is a reddening of some part of the oral mucosa, which is much more apparent during life than after death. This reddening usually begins in the floor of the mouth or the cheeks, or along the inner side of the upper lip. Lesions of greater severity show a more marked reddening and in addition raised areas of variable size and shape. The raised areas are either darker red than the surrounding tissue or of a greenish gray color. This appearance is due to superficial necrosis of the epithelium and the formation of a pseudomembrane on the surface, and for this reason is looked upon as a complicating lesion.

When the disease nears its natural termination the whole surface of the mouth and pharynx becomes deep red, obviously swollen and granular with greenish or grayish discolored areas. In dogs killed in the agonal stage of the disease the oral and pharyngeal mucosae are affected in their entirety and the lesion may extend into the esophagus. At this stage diffuse reddening is apparent in some areas but the major share of the surface is covered with gray or green pseudomembrane.

In dogs left to die there is found at postmortem an extensive superficial necrotic and diphtheritic condition of the upper alimentary tract.

Skin: Lesions of the skin of the scrotum were present in four dogs of this series. As seven of the thirteen dogs were males, and as similar lesions were observed in other dogs suffering from the disease experimentally induced by Goldberger and associates, but not studied in this series, the scrotal changes are not looked upon as accidental. The appearance of the scrotal lesion has been described by Goldberger and Wheeler;² it contrasts markedly with the normal, smooth, pale surrounding skin. No general cutaneous changes were observed in any of the dogs. No unusual tendency to shedding was apparent.

Intestines: Intestinal lesions were not observed in the earlier cases but in three later specimens the colon was thinner than normal, the inner surface was stained with reddish brown mucus and the mucous membrane generally was reddened.

Other Organs: No gross changes referable to the disease were observed in other tissues or organs. The bones were carefully examined.

Particular attention was paid to the periosteum of the long bones. It was uniformly of normal color and thickness. Periosteal hemorrhages were not seen.

The brain and cord were examined in each case but no gross changes were observed.

The characteristic gross appearances of the disease have been confined to the upper alimentary tract, colon, and skin.

HISTOLOGY

Mouth: The mouth lesions of the disease are readily accessible to observation during life and were naturally studied first. The development of the mouth lesions has been easiest to follow and observations made on them have been of material assistance in understanding the changes found in other tissues. They develop rapidly, change their character quickly, and thus the histology is more readily presented in narrative form. For the sake of brevity, reliance will be made on photomicrography rather than on detailed description.

The first changes appear in a narrow zone just beneath the epithelium and superficial to the subpapillary vascular plexus. Normally in the anterior portion of the dog's mouth the stroma just beneath the epithelium is compact, the vessels are small and inconspicuous, and there is but little difference in structure of the superficial stroma and the deeper fibromuscular tissue of the buccal wall. In the earliest recognizable lesions this zone becomes rarefied and unduly transparent as seen in microscopic preparations. The loss in density is due to the appearance of large intercellular spaces, widening of the vessels and loss of intercellular material — chiefly fine fibrillae. These changes are associated with redness of the mucous membranes seen during life.

The enlarged intercellular spaces were first looked upon as due to an edematous process, but this view has had to be revised because the process is not diffuse in the earliest lesions, and because the tissue spaces do not contain coagulable or stainable albuminous material. Edema fluid is readily coagulable with Zenker's solution and stainable with Giemsa's stain. Again, as the lesions spread and become more extensive the character of the intercellular material changes and it becomes coagulable with either Zenker's solution or formol. It is now thought that in the earliest phases of the lesions

the tissue spaces are filled with fluid too poor in albumen to be coagulable.

Particular effort has been made to understand the early degenerative changes in the fibrillae. It has not been possible to go beyond the observation that they become slender, shorter, and appear to fragment into converging rows of eosinophile dots. As these fibrillae are those which effect the attachment of the epithelium to the stroma, changes in the epithelium soon follow.

Alterations in the superficial vessels are as prominent as those in the fibrillar material of the stroma but they are secondary to them. The vessels become larger, their walls thin, and the endothelial cells long and slender. These changes give the appearance of a pathological vascularization of the stroma. They appear to result from degeneration of the supporting cells and fibrillae about the vessels and not to intrinsic changes in the walls. The impression is gained that the process is one of impaired vascular support rather than active engorgement.

The initial degenerative changes do not long remain in an uncomplicated state, for proliferation of perivascular cells soon becomes obvious and cells of the lymphocyte order appear in the intercellular spaces. So long as the epithelium remains intact migration of the polymorphonuclear leucocytes does not occur.

Degenerative changes in the covering epithelium appear clearly to be secondary to those in the stroma. The epithelial cells become pale, the intercellular spaces more readily visible, and the surface cells desquamate. The desquamation of the surface cells may reduce the epithelium to a single layer or complete denudation of the surface may result.

With loss in continuity of the epithelium the lesions assume a different appearance and become of a simple inflammatory type. A pseudomembrane forms on the surface made up of fibrin, cell debris and many forms of bacteria. Even when the surface epithelium is gone and the membrane has formed the lesions show but little tendency to suppurative reaction.

As shown in the summary statement in the appendix, several of the animals had had repeated attacks with recovery. In such cases active as well as partially healed lesions were found. Repair of the subepithelial stroma takes place by proliferation of connective tissue cells of larger size and with coarser fibrillae than those forming the

superficial stroma. It seems clear that the cells forming the stroma are more highly differentiated than most connective tissue cells and when destroyed are replaced by a simpler cell of the fibroblast type. The relations of the epithelium are not perfectly restored in repair but the stroma becomes simpler in structure with shorter and less highly developed vascular papillae. The more delicate vessels of the stroma are not replaced, but the coarser vessels of the subpapillary plexus come to lie closer to the epithelium.

Tongue: The dorsal surface of the dog's tongue is covered with coarse ridges of keratinized epithelium, which appear as curved barbs on section. These lie on a dense fibromuscular stroma. The inferior surface is covered with a thin epithelial coat and beneath this a stroma made up of delicate connective tissue. The difference in the structure of the stroma of the two surfaces appears to explain the well-nigh complete restriction of the lesions of the tongue to its inferior surface. The tongue lesions resemble those of the buccal mucosa and appear to pass through analogous phases.

Pharynx: The epithelium of the pharynx does not lie on the same type of stroma as in the anterior part of the mouth. A fairly broad zone of loose connective tissue containing lymphoid cells and small lymph follicles is interposed. For this reason rarefaction of the stroma is not so apparent, but even in early lesions the loss in density of stroma, fragmentation of fibrillae and the vascular changes previously described are visible. In more severe cases the epithelium becomes thin from degeneration and desquamation of cells and finally the stroma is denuded. Lesions of the pharynx appear to progress faster than those of the anterior part of the oral cavity. In sections including mucosa of both oral and nasal pharynx the lesions are confined to the hypopharynx.

Epiglottis: There is but little difference in structure of the upper and lower surfaces of the epiglottis. Lesions of the upper or pharyngeal surface are present in four cases, but they do not extend to the lower or laryngeal surface. The reason for this limitation in surfaces so contiguous is not clear.

Esophagus: Lesions of the esophagus are present in three cases and are similar to those of the buccal mucosa.

Respiratory Tract: In three dogs killed late in the series there are changes in the small bronchial radicles. The epithelium has lost its columnar character, the cells are enlarged and irregular in shape

and they fuse to form large giant cells about a central mass of mucus. The walls of these radicles are thin, the muscle coats are hyalinized and no lymphoid stroma remains between the epithelium and the supporting tissue. No other changes have been observed in the respiratory tract.

Stomach and Small Intestine: Comparison of sections from early and late cases show a progressive loss of density in the stroma of the villi. This appears to be due to the decrease in the lymph and reticulum cells. A colloid substance has diffused among the cells and fibrillae. This material while staining somewhat similar to mucus does not appear to be a product of the epithelium. Whether it is derived from the connective tissue of the stroma or is some plasma derivative is undetermined. Its distribution and appearance are similar to that of the intercellular material found in the subepithelial stroma of the mouth.

Large Intestine: In the later dogs of the series there are definite changes in the colon. The colic villi are distorted. Some are slender and appear compressed while those adjacent may be broad and bulbous. The lymphoid stroma is uniformly decreased. Between the villi are numerous mucus-filled cysts, formed by distention of crypts. In the broader villi the vessels are dilated and form small varices beneath the epithelium. There is great irregularity in the shape of the epithelial cells; some are oversized and triangular in shape, others are flat and elongated, while those lining the small cysts in the mucous membrane are stretched and distorted by distension.

Skin: Changes analogous to those observed in the buccal mucous membrane are present in sections of the skin from the scrotum. In three cases the corium shows the same rarefactive changes seen in the buccal lesions. A late case shows repair of the superficial collagen by proliferating fibroblasts. As in the mouth the process appears to begin in the fibrillar material supporting the epithelium.

Nervous System: No definite changes have been observed in the central nervous system. The number and arrangement of the tigroid granules in the large ganglion cells of the brain and cord are apparently subject to some variation normally. No striking change in the tigroid substance or in the nerve cells has been found in the animals of this series.

Other Organs: Nothing of significance has been found in the other tissues or organs of the animals examined.

CONTROLS

As shown in the appendix, Dog 100 was killed at the outset of the experiment as a normal control. Additional normal material was obtained from animals provided by the Department of Health at Yonkers, New York. Histological appearances comparable to those described have not been seen in the normal animals.

Dog 76, suffering from a polyneuritic condition induced by a deficient "synthetic" diet was extremely emaciated and partially paralyzed. Mucous membrane and cutaneous lesions were not observed clinically and histological preparations show no special changes in these tissues. Sections of the spinal cord stained by Weigert's method show a degenerative process in the pyramidal tracts.

SUMMARY

The lesions of experimental black tongue are located in the mucous membranes of the mouth, pharynx, esophagus, intestine, and skin of the scrotum. They originate in a degenerative process affecting the superficial connective tissue of the mucous and dermal membranes of these respective surfaces. Changes in the supporting tissues of these mucous membranes are followed by secondary ones in the epithelium. The lesions tend to terminate in an extensive necrotic and diphtheritic inflammation of the upper alimentary tract.

DISCUSSION

On account of the similarity in the appearance clinically of the mouth and skin lesions of experimental black tongue to those of pellagra in man (Goldberger and Wheeler) it may be of value to compare the microscopic features of the lesions of the two diseases.

The pellagra material available for comparison was collected by the present writer in Panama in 1923 and described in 1924.³ It has been completely restudied in connection with the present investigation, but no important additions can be made to the observations originally reported. The special value of this human material lies in the fact that the cases studied were typical and uncomplicated cases of pellagra. The patients were admitted to the hospital suffering with pellagra and four died while still in good stages of nutrition. The salient features of the observations reported may be briefly summarized as follows:

Skin: The early skin lesions in the stage of redness and swelling, usually referred to as the erythematous stage, owe their peculiar characters not to acute inflammation (in the ordinary sense of the term) but to a rarefactive process in the superficial corium and to widening of the superficial vascular channels. The earliest tissue change is a disintegrative process affecting the fibrillar structures which constitute the binding substance between the corium and epidermis. This process may be so complete as to bring about separation of the epidermis over extensive areas. In milder lesions the disintegrative process and separation of the epidermis may be confined to microscopic areas. The thickening of the skin and the scaling in the later cases is due to abortive or imperfect repair of the corium. Healed lesions usually show atrophy of the skin with marked thinning of the epidermis and often with telangiectases in the cicatrized corium. The skin lesions show no evidence of being infectious in origin and often remain aseptic throughout their course.

Mouth, Pharynx and Esophagus: The remarkable feature of the lesions of the upper alimentary tract is their similarity to the skin lesions. They have their basis in degenerative changes in analogous fibrillar and cellular structures. The striking red color of the mouth and pharynx is perfectly analogous to the erythema of the skin. The fact that the mouth lesions do not remain aseptic is responsible for the apparent differences in the lesions.

Intestine: Lesions of the intestine, most marked in the colon, while not bearing superficially a resemblance to those of the mouth and skin, are, on analysis, quite similar. The supporting elements of the mucous membrane are the structures primarily affected. Secondary, degenerative changes in the epithelium result in denudation of the stroma with the formation of a diphtheritic membrane. Small diphtheritic lesions tend to spread and terminate as larger and deeper ones. In later cases the colon may show marked atrophic changes with reversion of the mucous membrane to a simpler type, often with small mucous cysts in the mucosa. Telangiectases like those in the corium are found in the colonic mucosa in later cases.

Nervous System: No distinctive changes are found in the central nervous system in the earlier cases. In the later ones in which there had been repeated attacks and in which the patients died in an emaciated stage there are hyaline changes in the small vessels and chromatolytic changes in the ganglion cells of the brain and cord.

In general the alterations in the central nervous system are in inverse proportion to the acuteness and extent of the lesions of the skin and alimentary tract.

It appears that entirely too much significance has been attached in the literature to the rather indefinite changes described in the nervous system, especially since definite information is lacking as to the permanence of the alterations in the chromatic substance of the nerve cell to which the term chromatolysis has been applied. The majority of the studies of the central nervous system in pellagra have been based on material collected in insane asylums.

COMPARISON OF THE LESIONS OF PELLAGRA WITH THOSE OF EXPERIMENTAL BLACK TONGUE

The lesions of the skin, mouth, pharynx, esophagus and colon in pellagra and in experimental black tongue in dogs show very similar gross appearances. Histologically the lesions of both appear to have their inception in a degenerative process in an analogous tissue element. The processes of repair in both result in fibrotic replacement and in pathological vascularization of the superficial stroma of the mucous membrane of the upper alimentary tract and of the corium. The lesions of both are primarily degenerative in character and have the same tendency to secondary infection.

The lesions in the experimental condition in the dog render the lesions of pellagra more understandable because the former can be obtained earlier and are less complicated by infection than in man.

The distinctive lesions of pellagra and those of black tongue of dogs appear to have their origin in a failure on the part of the organism to maintain the specialized supporting tissues of epithelium in various situations.

APPENDIX

Summary of significant details in the history of each of the dogs from which tissues were secured. Furnished by Surgeon, Joseph Goldberger, U. S. Public Health Service

Dog 13: Male. Between April 7, 1923, when acquired, and April 28, 1927, when killed, had in all eight attacks of experimental black tongue. The last attack began January 25, 1927, or 48 days after beginning the experimental diet. This was a relapsing attack accompanied by self-imposed semistarvation. Weighed 9.4 kilograms when last attack began, and 6 kilograms 2 days before he was killed. Had an intercurrent infective ulcerative stomatitis between March 15 and April 17, 1927.

Dog 15: Male. Between April 14, 1923, when acquired, and July 12, 1927, when killed, this animal had in all four attacks of experimental black tongue. The last attack began June 28, 1927, that is 117 days after beginning the experimental diet. This was a relapsing attack of moderate severity. Killed with illuminating gas, July 12, 1927.

Dog 42: Male. Between June 26, 1923, when whelped in the laboratory, and October 16, 1926, when killed, had in all four attacks of experimental black tongue. The last attack began September 28, 1926, or 56 days after beginning the experimental diet. In dying condition was killed with illuminating gas. Weighed 12.8 kilograms when last attack began, and 11.5 kilograms 4 days before he was killed.

Dog 62: Male. Whelped in the laboratory November 4, 1923. Up to June 12, 1926, had had no recognizable black tongue. In good condition June 12, 1926, when experimental diet was begun. Killed with illuminating gas June 26, 1926. No signs of black tongue during the observation period of 14 days.

Dog 66: Male. Between November 25, 1923, when whelped in the laboratory, and January 18, 1927, when killed, had in all four attacks of black tongue. The last, a chronic, mild, relapsing attack with a flaccid paraplegia accompanied by semistarvation began December 3, 1926, about 10 months after beginning an experimental diet. Weighed 9.8 kilograms on November 30, 1926, 2 days before the beginning of the last attack, and 7.5 kilograms on day when killed with illuminating gas.

Dog 69: Male. Whelped in the laboratory November 25, 1923. Developed the first and only attack of experimental black tongue July 28, 1926, or 46 days after beginning the experimental diet. Killed with illuminating gas on August 7, 1926, on the 10th day of a mild developing black tongue.

Dog 76: Male. Between June 9, 1924, when acquired, and June 29, 1927, when killed, had in all one relapsing attack of experimental black tongue, which began September 4, 1926, from which he fully recovered. On December 8, 1926, began an antineuritic deficient "synthetic" diet. On January 27, 1927, that is, at the end of 50 days, this animal developed signs of "polyneuritis." Two days later, when in dying condition, was killed with illuminating gas.

Dog 82: Bitch. Between October 13, 1924, when acquired, and January 30, 1927, when this animal died, she had had in all three attacks of experimental black tongue. The last attack began January 22, 1927, or 45 days after beginning the experimental diet; a rapidly progressive attack. The animal died sometime between 9 P.M., January 29, and 1 A.M., January 30, 1927.

Dog 100: Male. Whelped in the laboratory December 9, 1925. Reared on a stock diet. Normal animal. Killed with illuminating gas, June 26, 1926.

Dog 101: Male. Whelped in the laboratory December 9, 1925; litter mate of Dog 100. Reared on stock diet. In good condition June 12, 1926, when the experimental diet was begun. First sign of a mild relapsing progressive attack appeared July 10, 1926. Killed with illuminating gas August 7, 1926.

Dog 102: Male. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. First sign of

a moderately severe, rapidly progressive attack of experimental black tongue appeared July 10, 1926. Killed with illuminating gas July 20, 1926.

Dog 103: Male. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. First sign of relapsing, moderately severe attack of experimental black tongue appeared July 10, 1926. Killed with illuminating gas August 7, 1926.

Dog 104: Bitch. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. Mild beginning experimental black tongue, July 6, 1926. Killed with illuminating gas July 10, 1926.

Dog 105: Bitch. Litter mate of Dog 100. Reared on a stock diet. In good condition on June 12, 1926, when the experimental diet was begun. A mild relapsing experimental black tongue began July 15, 1926. Killed with illuminating gas August 7, 1926.

Dog 106: Bitch. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. Killed with illuminating gas June 26, 1926. No signs of black tongue during the observation period of 14 days.

Dog 109: Bitch. Acquired October 29, 1926. A fatal attack of experimental black tongue began December 30, 1926, 22 days after beginning the experimental diet. The attack was a relapsing one accompanied by prolonged self-imposed semistarvation. Weighed 7.9 kilogram on December 28, 1926, and 3.4 kilograms on April 19, 1927. Died April 20, 1927.

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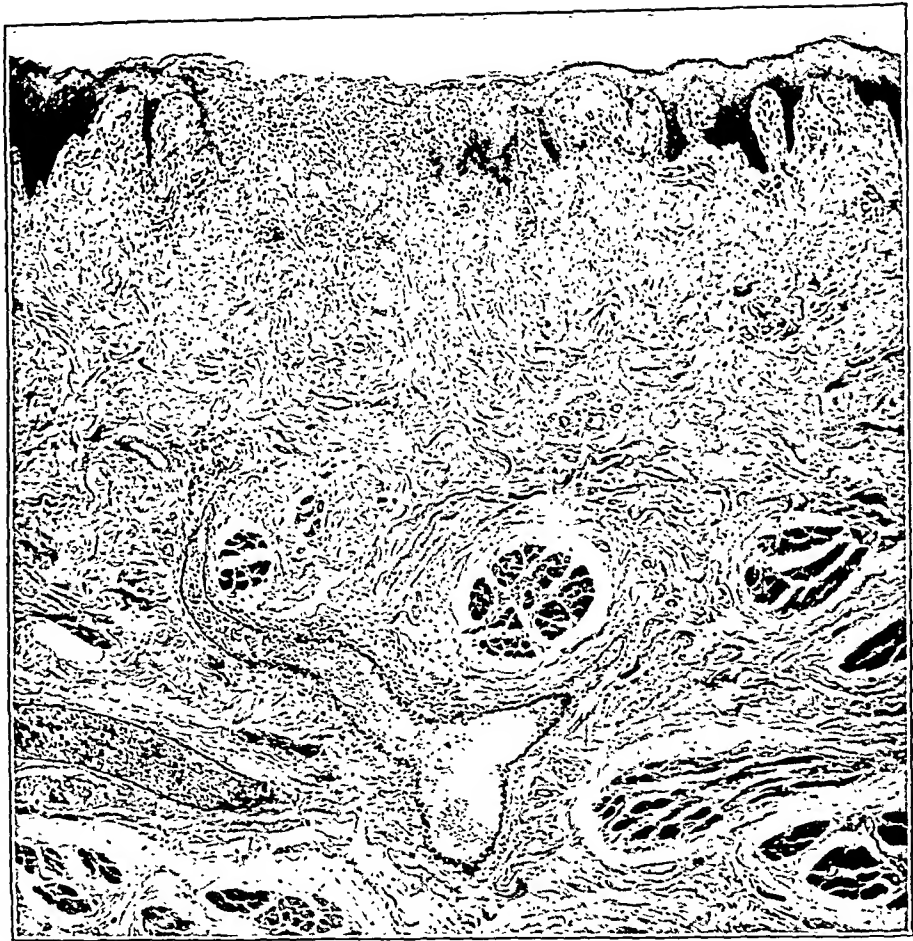
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DESCRIPTION OF PLATES

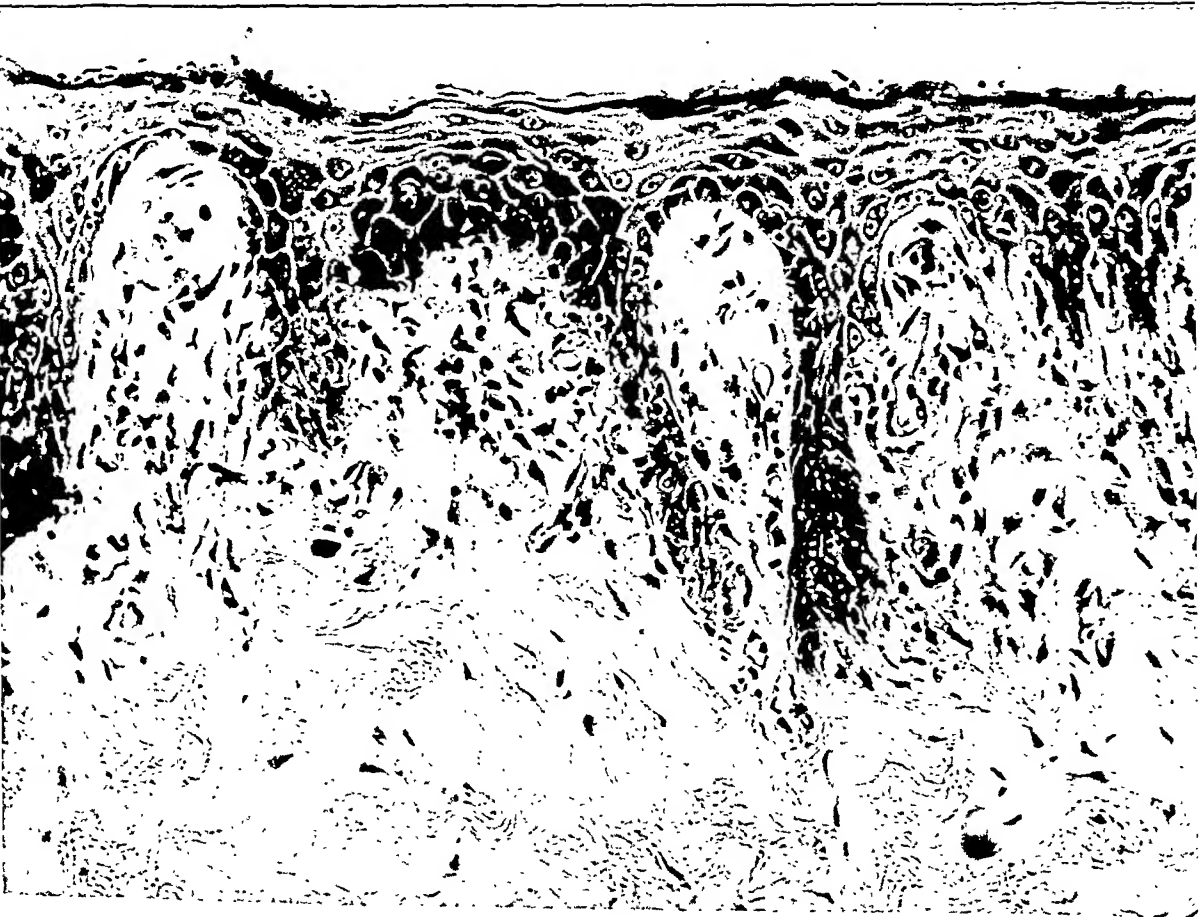
PLATE 80

FIG. 1. Dog 101. Buccal mucosa, margin of an early lesion. Rarefaction of the subepithelial stroma and degenerative changes in the epithelium.

FIG. 2. Dog 104. Buccal mucosa, early lesion. Fragmentation of the fibrillar material contiguous to the basal layer. Note the very limited changes in the epithelium.



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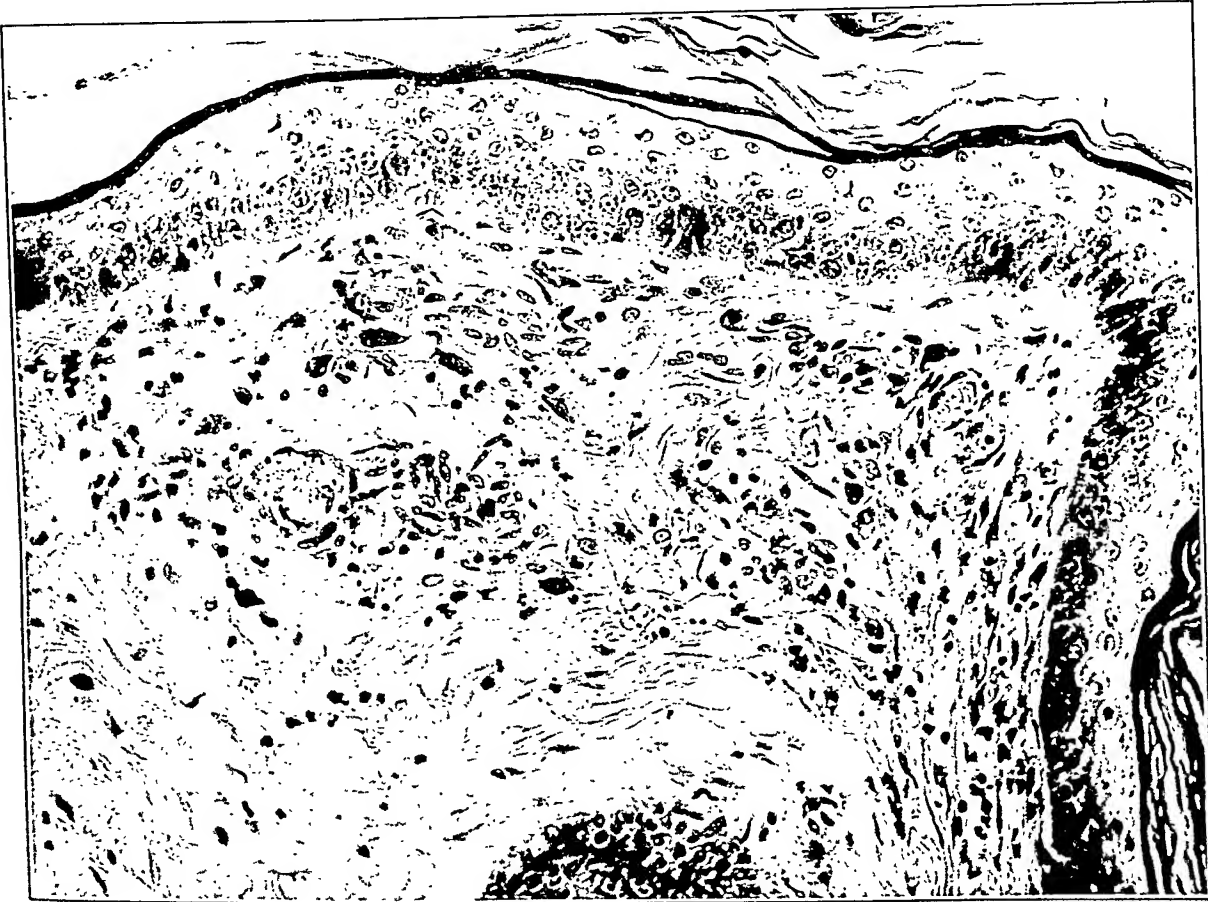
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PLATE 81

- FIG. 3. Dog 103. Skin, scrotum. Changes in the corium similar to those in the subepithelial stroma shown in Fig. 2.
- FIG. 4. Dog 15. Skin, scrotum. Rarefaction of the corium and degeneration of the superficial fibrillae.



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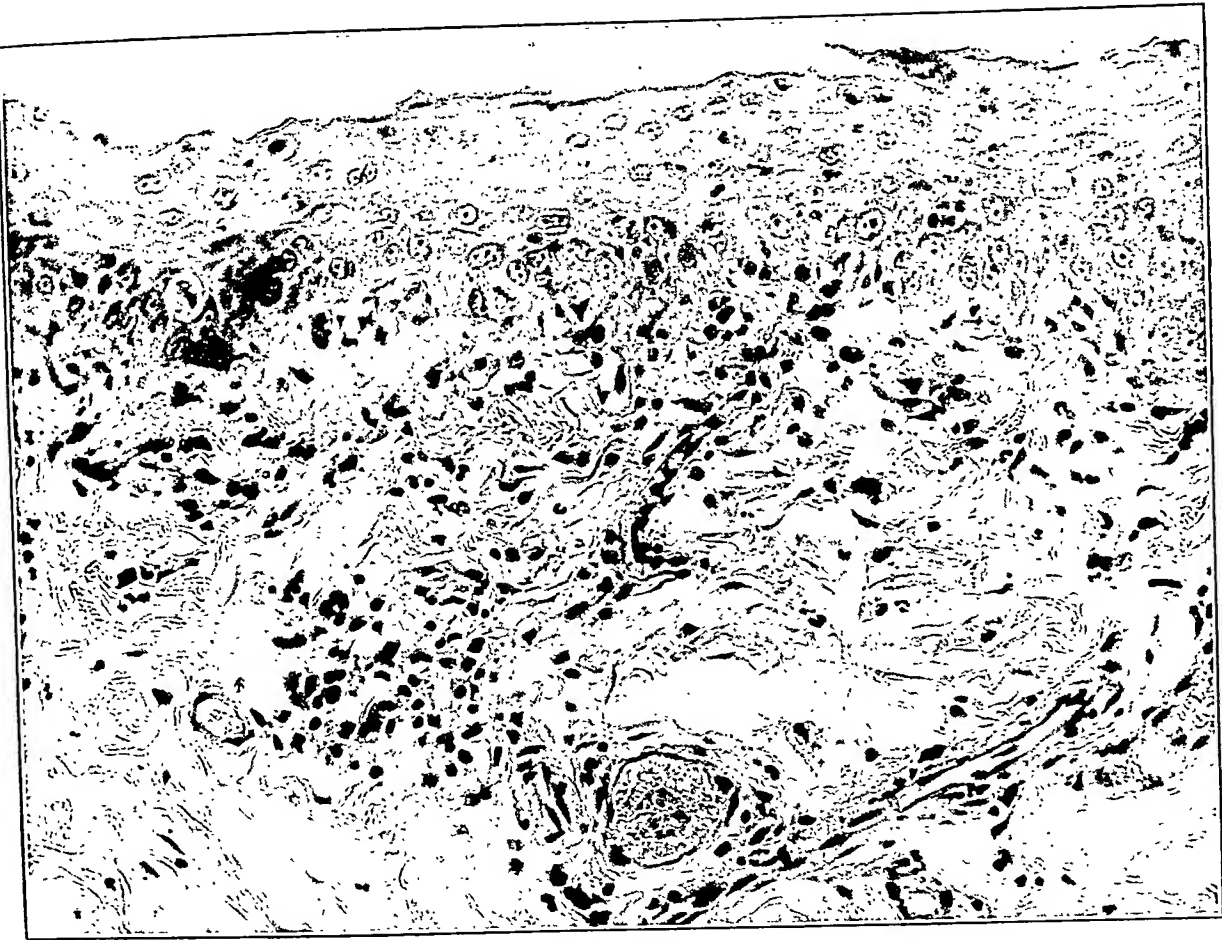


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PLATE 82

FIG. 5. Human skin. Skin from pellagra in man. Note the similarity in the changes in the corium to those in the buccal mucosa and in the skin of the scrotum in the dog.

FIG. 6. Dog 109. Colon, late lesion. Aplasia of the villous stroma, regressive changes in the intestinal epithelium with formation of mucous cysts in the dilated glands.



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6

Denton

Experimental Black Tongue of Dogs

OBSERVATIONS ON BLOOD INCUBATED UNDER ABNORMAL CONDITIONS *

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In two previous papers^{1, 2} we have reported our observations on incubated leukemic and normal blood. In the experiments described below, we studied the effect of various substances and different cultural conditions on the leucocytes of incubated normal human and rabbit blood. In our preceding papers, we described the development of two types of large cells in both leukemic and normal blood. We termed these two types of cells "X" and "Y." The "X" cell was thought to be derived from a pluripotential, embryonic blood cell, while the "Y" cell developed from the monocyte. This brief mention of these cells is made here as the terms are used in certain experiments in this paper. We have described in detail in our other work the terms "monocyte," rosette," etc.

METHODS

Blood, removed from the median basilic veins of humans or from the hearts of rabbits, was placed in small tubes and allowed to clot, or it was added to tubes containing sodium citrate or heparin and then treated in different ways described later. In every experiment the tubes were sealed with paraffin before incubation. Specimens for examination were removed with a platinum loop at various times. These specimens were studied by the supravital technic of Sabin *et al.*, and in air-dried smears by Wright's stain and Sato and Sekiya's peroxidase reaction.

OBSERVATIONS

Each single experiment in the various series will not be gone into in detail but a composite description of the results in each group will be given. The heading of each set of observations indicates the substance or condition the effect of which was being studied.

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ICEBOX TEMPERATURE

In the experiments previously reported the bloods were incubated at 37.5°C . The question arose as to the significance of the large cells that appeared. Were they merely degenerating forms or were they the result of physiological activity? If they were the former, it seemed probable that they would appear under conditions where the leucocytes could be kept alive for a period of time with their physiological activities diminished or suppressed. Icebox temperature seemed to offer such conditions, for others have shown that tissues can be grown after being kept in the icebox for at least eight days. Accordingly experiments were set up with clotted rabbit blood, one set of tubes being kept at 10°C , another set as controls at 37.5°C . The results of the tubes kept at 10°C were as follows: Leucocytes of the different types were found alive at the end of three weeks but not four weeks. From the sixth day on an increasing number of dead cells of various kinds were found. The polymorphonuclear neutrophils as time went on contained globules staining with neutral red, situated at the periphery of the cells. The lymphocytes remained normal in size but there was some increase in the size of their neutral red granules. The monocytes likewise showed no change in size but their rosettes became larger due to the increased size of the neutral red granules. No refractile granules, which were so commonly seen in these cells at incubator temperature, were noted. No large cells of any type or transition forms were ever found. In one experiment, a tube that had been incubated eleven days at 37.5°C and that contained a considerable number of large cells was placed at 10°C and observed for six days. The cells remained alive and showed no change in their morphology or size. No tendency to revert to the forms from which they were derived was noted.

The results of these experiments would seem to confirm the view that the large cells are the result of physiological activity and are not merely degenerative forms.

SODIUM CITRATE AND HEPARIN

One of the difficulties we encountered in working with clotted blood was the scarcity of leucocytes in our preparations during the first few days of incubation. As time went on, leucocytes appeared in larger numbers. Apparently the cells were caught in the clot at

the beginning and gradually migrated out into the serum. To obviate this difficulty we decided to use an anticoagulant, sodium citrate. This was employed at a concentration of 1 per cent in Locke's solution and an equal volume of blood was added. For the first few hours after the addition of blood to this solution, the leucocytes were rounded up and non-motile. After twenty-four hours' incubation they had regained their normal appearance and motility. However, after forty-eight hours they were all dead, showing a peculiar ballooning of their cytoplasm. It seemed as if it took the leucocytes some time to adjust themselves to this solution, but even after they had done so they could live but two days. If the citrated blood was centrifuged shortly after citrating and about two drops removed from the upper layer of the sediment and added to 1 cc. of serum, the cells survived for long periods with the development of many large cells. However, clotting occurred under these conditions and made observation difficult.

After our experience with sodium citrate, we decided to try heparin, which has been so useful in physiology and for tissue cultures (Craciun³). Heparin was dissolved in Locke's solution and sterilized at fifteen pounds pressure for fifteen minutes in the autoclave. At first, we used final dilutions of 1:15,000 as recommended by Craciun for heparinized plasma but we found that such a concentration did not prevent the blood from clotting. Eventually we found that a final dilution of 1:600 or 1:800 was satisfactory for human blood. Rabbit blood always clotted at such dilutions. The fact that whole blood will clot at concentrations of heparin that prevent clotting of plasma alone, suggests that the cells of the blood must yield substances causing clotting similar to those of tissue cells. The presence of heparin did not affect either the periods of survival of the various types of leucocytes or the development of the large cells.

OLD CULTURE FLUIDS

Each type of leucocyte had a rather definite period of survival in our experiments on incubated blood. Also large cells made their appearance at about the same time in each experiment. The question naturally arose: Was the length of life of the leucocytes and the development of the large cells dependent on the accumulation during incubation of waste products in the medium? If this were the case,

then renewal of the fluid medium should prolong the life of the cells, and conversely, the use of fluids in which cells had been incubated should shorten the life of the cells. Also such changed conditions should affect the development of the large cells. In several instances, therefore, we removed the fluid after centrifuging the tubes slowly, and added fresh fluid of the same composition. The results were inconclusive; no definite effect could be noted. We then tried the other method of attack. Tubes that had been incubated respectively eight days and two weeks were centrifuged. The supernatant fluids were removed and added to equal volumes of fresh, heparinized human blood. The blood in all sets of tubes was taken from the same individual. These supernatant fluids were dark brown-red owing to hemolysis. After twenty-four hours incubation, the monocytes and also some of the polymorphonuclear neutrophils contained many granules of what appeared to be hemoglobin. (Phagocytized granules of similar appearance were often seen in cells in the usual incubated blood tubes after a weeks incubation when considerable hemolysis had taken place.) The monocytes were either oxidase-positive or negative, whereas in the control tubes all the monocytes were oxidase-positive. The general condition of the cells was about the same as in the control tubes. Incubation was continued for three weeks. The different types of leucocytes survived the same periods of time as in the controls. These different periods were the same as those we have found in our other experiments. "X" cells appeared earlier and occurred throughout in greater numbers than in the controls. There were also more, "Y" cells than in the controls.

From the result of these experiments, it would appear that the death of the cells under the conditions of our cultures was dependent on their natural length of life, not on the accumulation of waste products, and the presence of old culture fluids favored the development of "X" and "Y" cells. In a way our results agree with those of Awrorow and Timofejewskij.⁴ These workers found in culturing leukemic blood that subcultures to fresh medium did not prolong the life of the cells.

LITHIUM CARMINE AND TRYPAN BLUE

These experiments were carried out with clotted rabbit blood. A few drops of 5 per cent lithium carmine or 1 per cent trypan blue

were added to the tubes after the blood had clotted. In one experiment, a rabbit was bled five minutes after receiving intravenously 5 cc. of unfiltered lithium carmine solution. The serum of this specimen was highly colored with the dye but there was no carmine in any of the leucocytes. Tubes in each series, with and without the dyes, were incubated at 37.5°C and specimens were studied on both unstained slides and slides with neutral red.

The dyes had no apparent effect on the duration of survival or development of the cells. Large cells appeared in approximately the same number and of the same morphology as in the controls. In one experiment, there was a suggestion that the cells in the tubes containing the dyes were more phagocytic but this may have been chance and it was noted in only this one experiment. Neither carmine nor trypan blue was taken up as such in any appreciable amount by any type of cell. When examined on unstained slides, the large cells sometimes showed some fine granules of dye in their rosette areas. The dyes stained dead cells, and such stained dead cells and débris were frequently found in the large cells. Whether this phagocytosed material was stained before or after phagocytosis, it is impossible to say. Granules or masses of dye, except those mentioned above, were never found in the cells. In an experiment carried out at 10°C with carmine, no dye was found in the cells nor had it changed the cells in any way.

HIGGINS' AND WEBER'S INDIA INKS

Muller⁵ has recently described the effect of repeated injections of these two inks. There was a striking difference in the results with the two inks which apparently was dependent on the protective colloid present, not on the carbon. We felt it would be of interest to compare the two inks in their action on the development and survival of the large cells of incubated blood. Clotted rabbit blood, 2 cc. to a tube, was used and one loopful of ink was added to a tube. Controls without ink were always run. The results were striking. Large cells developed normally in the presence of Higgins' ink whereas no such cells were found in the tubes containing Weber's ink. The differences in the effects of these two substances was probably to be ascribed to the particular protective colloid in Weber's ink. Higgins' ink occurred in the large cells both as very

fine granules in the rosette area and in larger masses at the periphery of the rosette. Such ink rosettes on an unstained slide simulated closely in size, number and arrangement of granules those stained by neutral red. Observed on a neutral red slide, the ink in the rosette area was slowly replaced by the neutral red until finally no trace of the ink could be seen. This bringing out of rosettes by Higgins' ink was observed in another kind of experiment. In this, a drop of diluted ink was added on a slide to a drop of incubated blood that contained many large cells. After four hours' incubation, the rosettes of the large cells were delineated clearly by the ink particles, and were identical in appearance with rosettes stained by neutral red. It would seem, therefore, that certain colloidal solutions such as Higgins' ink, and to a less extent carmine and trypan blue, occur as fine particles in the rosette areas, possibly deposited on the same granules of the cells that neutral red stains.

CARBON

We hoped by allowing cells to phagocyte carbon and then doing an oxidase reaction to be able to distinguish two types of phagocytic mononuclear leucocytes — one oxidase-positive, the other oxidase-negative. Carbon, in the form of lampblack, was added to heparinized human blood. The tubes were incubated at 37.5° C. After two hours incubation, some of the polymorphonuclear neutrophils and monocytes contained granules of carbon. After four hours, the polymorphonuclears had phagocyted a little more carbon. The monocytes contained varying amounts of carbon. Where it was small in amount and in fine granules, it occurred at the periphery of the rosette. When the amount was larger and the granules coarser, no rosette could be seen. In some of these cells, a few fine neutral red granules could be made out above or below the nucleus, but in others no neutral red granules could be found in any part of the cell. When examined with the oxidase reaction, the monocytes with a few carbon particles were found to be all oxidase-positive. Other monocytes that contained more carbon had fewer oxidase granules and some that contained the most were apparently oxidase-negative. In other words, the number of oxidase granules present was in inverse proportion to the amount of carbon in the cells. The oxidase reaction paralleled closely the neutral red picture, *i.e.*, the more the phagocyted carbon particles, the fewer the stainable granules.

Stained by Wright's method, practically all the monocytes contained carbon. There was a marked tendency for these cells to occur in clumps around large masses of carbon. At twenty-four hours, in the supravital preparations, the monocytes were grouped around carbon masses. Their rosettes were obscured or absent. The phagocytosed carbon tended to be at the periphery of the cell if there was not too much present. Some of the finest granules occurred in the rosette areas. In preparations stained with both neutral red and Janus green, no mitochondria could be seen in the monocytes although numerous in the lymphocytes. In the fixed smears, all monocytes contained carbon and all were oxidase-negative. At three days, most of the monocytes were dead while at four days they were all dead. The other types of leucocytes were living and normal and survived their usual periods. A few "X" cells developed, but no "Y" cells.

We have described the above in such detail because it brings out two points of importance. The first is the apparent or real loss of the monocytes' neutral red rosettes, so characteristic of this cell, following the ingestion of carbon. The second is the change in the oxidase reaction from positive to negative in these phagocytic monocytes.

In short, after taking up carbon, the monocytes lost the two characteristics by which they are recognized — rosette formation and oxidase reaction. The importance of the possibility of such changes is obvious, for one could easily be misled in attempting to identify such cells without having followed them from the beginning. Their negative oxidase reaction, lack of rosette and marked phagocytosis would place them in Sabin's clasmatocyte or endothelial cell group, a type of cell held by her school to be quite distinct from the monocyte group.

TUBERCLE BACILLI

Some living avian tubercle bacilli were added to clotted rabbit blood tubes. Large cells had developed at five days and they contained bacilli. The rosettes of the large cells tended to be somewhat broken up and irregular. The bacilli occurred between the neutral red granules at the periphery of the rosette and were often arranged radially with respect to the centrosphere. These cells remained alive but half as long as those in the controls. Living tubercle bacilli,

human strain H 37, were added to heparinized human blood. After five hours incubation, the monocytes were found grouped about clumps of bacilli. The granules of the rosettes of these cells were larger than normal and somewhat irregular in size. The other type of leucocytes appeared normal. At twenty-four hours, a stain for tubercle bacilli followed by Wright's stain showed the monocytes containing masses of tubercle bacilli and a few of the polymorphonuclear neutrophils with single bacilli or small clumps. In both the supravital and Wright's stain, the monocytes looked in poor condition while the other leucocytes appeared normal. The monocytes were oxidase-positive. All the monocytes were dead at three days. The polymorphonuclear neutrophils and eosinophils lived seven days. The lymphocytes could be followed twelve days and they remained unchanged in appearance during this time. The bacilli grew freely in the fluid. No large cells developed.

FOREIGN ERYTHROCYTES

Phagocytosis of red cells by "X" and "Y" cells was a common phenomenon in incubated blood. The red cells were sometimes taken up unchanged. At other times, phagocytic cells were found containing eight to twelve completely hemolyzed erythrocytes. Whether hemolysis took place before or after phagocytosis is difficult to say, but probably the former was usually true, for at such times practically all the red cells in the tubes were completely hemolyzed. Also, it was rare to find cells containing so many unhemolyzed red cells. In some experiments designed to study the production of antibodies by the blood leucocytes, sheep blood was added to incubated blood tubes at various times. Phagocytosis of these foreign red corpuscles was often active. Such phagocytosis was also used by us as a test for the physiologic activity of the large cells. Such a test had significance as it was possible that the large cells were inactive, degenerating forms. In one such instance, a small amount of sheep blood was added to tubes of rabbit blood that had been incubated eleven days and contained many large cells. Twenty-four hours later, the tubes were examined and showed marked phagocytosis of the sheep red corpuscles by the large cells. Some contained as many as thirty red cells. The method of phagocytosis was observed in the supravital preparations. The peripheral portion including the pseudopods

of the large cells consisted of clear, non-granular cytoplasm. A red cell would be taken into this clear portion and moved up to the edge of the granular area. At this point, the red corpuscle was either taken into the granular region and placed at the periphery of the rosette or it was violently expelled out of the cell into the surrounding fluid. Cells were seen to take up and reject several red cells in succession before one was taken into the granular area. Some large cells were observed to admit to the granular area as many as three red cells at the same time. It seemed as if the red cells were taken into the clear cytoplasm tentatively. Then some factor decided whether they should be taken up permanently or whether they should be expelled immediately. Erythrocytes never remained any length of time in the clear zone.

SUMMARY

The effect of the addition of various substances on the physiologic activities and development of large cells in incubated normal rabbit and human blood is described. These substances included carmine, trypan blue, Weber's and Higgins' inks, carbon, tubercle bacilli and old culture fluids. The periods of survival and changes of the different types of leucocytes at 10° C are reported. The use of citrated and heparinized plasma and their effect on leucocytes are discussed.

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TWO OSTEOLASTOMAS NOT CONNECTED WITH BONE, HISTOLOGICALLY IDENTICAL WITH OSTEOLGENIC SARCOMA, AND CLINICALLY BENIGN *

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In the eight years which have elapsed since the establishment of the Bone Sarcoma Registry by Codman the interest of both pathologists and clinicians in tumors of bone has been steadily increasing. Due to the pioneer work of Codman and the recent review of Kolodny¹ a great mass of well organized and classified material is now available.

During the past two years two patients have been under treatment at the Boston City Hospital who have had tumors in the thigh not attached to bone. Microscopic examination has shown the tumors fulfil every criterion used for the establishment of a diagnosis of osteogenic sarcoma. They have been completely removed surgically and now at the end of two years for one, and six months for the other, there has been no evidence of recurrence.

The diagnosis of tumors of the type of osteogenic sarcoma is often based on the histologic findings. When such a diagnosis is made the prognosis for the patient is so bad that we have felt that these two cases deserve to be put on record.

CASE I. *Clinical History:* The patient, S. L., female, unmarried, a department-store worker, 21 years of age, entered the hospital August 17, 1926, under the care of Dr. Robert Cochrane. She complained of a lump on the right thigh of five weeks duration. The family history, past history and habits were irrelevant.

The present illness began about five weeks before her admission to the hospital. The patient was in the habit of carrying a pocket filled with coins. As she walked, this heavy weight repeatedly struck her right thigh. Her attention was first called to that region of her body by the development of a slight pain felt only when she moved her leg. On examination she felt a firm, slightly tender mass about the size of an English walnut in the location which was struck by the coins. She thought that there had been a definite increase in the size of the tumor in the time which elapsed between her first observation of it and her

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entrance to the hospital. Except for the loss of about ten pounds in weight during the previous six months she had noticed nothing wrong and had felt perfectly well.

Physical examination showed a fairly well developed and well nourished girl in no distress. The head, chest, neck, arms and abdomen were negative. No lymph nodes were palpable. Examination of the bones revealed no abnormality. On the inner, upper aspect of the right thigh over the adductor group of muscles there was a firm, oval mass about 5 cm. in the greatest diameter. The tumor was very indurated, discrete and firmly attached to the underlying structures. No acute tenderness or fluctuation was observed. Slight pain could be elicited by deep palpation. The skin over the mass was not abnormal.

X-ray studies of all bones were negative.

The preoperative diagnosis was "bone tumor."

Operation was performed under gas-oxygen anesthesia. The skin and subcutaneous tissues were retracted and a tumor mass about 5 cm. long was displayed. It was oblong in shape, very firm, an even gray in color, and firmly attached to the adductor magnus muscle. The mass was dissected free and shelled out very easily. Little bleeding was encountered.

The postoperative course was uneventful and the patient was discharged on the ninth day.

Complete physical examination and X-ray studies of the bones, done two years after operation, show no evidence of recurrence. The patient has been perfectly well and active.

PATHOLOGIC REPORT

Gross Description: The specimen consists of an irregularly oval mass of pink tissue with a rough surface. Consistence is very uniform and hard. Cut section is pink, mottled with gray translucent areas. Considerable calcification is present.

Microscopic Examination: The background of the tumor consists of narrow, elongated, spindle-shaped cells, mostly of a fairly adult type with an oval nucleus and a small amount of acidophilic cytoplasm. The nuclear chromatin is small in amount and rather scattered. In the preparations stained with phosphotungstic acid-hematoxylin the fibroglia fibrils are very clearly seen. The intercellular substance is composed of delicate collagen fibers grouped in parallel rows to form wavy strands. There is a tendency to form bundles which run at right angles to each other. There are a few fat cells and occasional groups of lymphocytes present. In certain areas every stage in the transition from connective tissue to osteoid tissue and from osteoid tissue to bone may be seen. On the collagen between the connective tissue cells, strands and small masses of smooth brown-staining material are laid down. These strands anastomose and the size increases. The fibroblasts in these regions

have become enlarged and are quite variable in appearance. They have lost their fibrils and have become oval to fusiform in shape. The cytoplasm is greater in amount and often is basophilic. The nuclei are larger, more irregular in size, and contain a large amount of chromatin. Mitotic figures are quite plentiful although no multiple mitoses are seen in this tumor. As the intercellular substance increases in amount single and paired cells are isolated and appear in small lacunae. As the bone more nearly approaches the adult type the intercellular substance takes a brighter pink with eosin and the cells decrease in size to appear like typical bone cells. Around the bone spicules rows of osteoblasts are arranged laying down more hyaline substance. A fair number of small, well formed vessels are present. The appearance of the bone is variable. It is for the most part unorganized, atypical, and of neoplastic type, although a small amount of normal-appearing adult bone is seen. Foreign body giant cells are present in small numbers. No muscle fibers are seen.

CASE II. *Clinical History:* The patient, L. K., a married business man, 37 years of age, entered the hospital December 2, 1927, complaining of a swelling in the left groin, of one years duration. The family history, social history, past history and habits were irrelevant to the present illness.

The condition began twenty-eight months before admission to the hospital when he struck his left groin on the sharp corner of a piece of furniture. A tender, bruised area resulted which subsided after several weeks without leaving signs or symptoms. About fifteen months before admission he first noticed a firm lump about 1 cm. in diameter on the inner side of the upper left thigh at about the same location as the previous bruise. The mass gradually grew larger but caused no discomfort until three weeks before entry when he noticed that pressure on it caused slight pain. He felt perfectly well and had no discomfort.

Physical examination showed a well developed and well nourished man in no distress. The head, neck, arms, chest and abdomen were negative. Both testicles were in the scrotum and were negative to palpation. The only lymph node palpable was one about 4 mm. in diameter in the left inguinal region, which was not adherent or tender. Examination of all bones was negative. On the inner aspect of the upper left thigh there was an extremely hard, smooth, rounded mass measuring roughly 2 by 2 by 6 cm. It was quite superficial and not adherent to skin or underlying tissues. The skin over the mass was normal in appearance.

Examination of the blood and urine showed nothing abnormal. Blood Kahn and Wassermann reactions were negative.

X-ray films of the chest and all the bones of the skeleton showed no abnormality. X-ray examination of the tumor showed an area of increased density corresponding in size to that of the tumor mass itself.

The provisional diagnosis was fibroma of the fascia.

Operation was performed by Dr. Donald Munro, December 4, 1927, under local anesthesia. The mass lay just under the skin in no way connected with underlying muscle or deeper tissues. Surrounding tissues were readily peeled off and no evident blood vessels leading to the tumor mass were seen. The wound was closed without drainage; the postoperative course was uneventful. No evidence of recurrence has appeared up to the present, six months after operation.

PATHOLOGIC REPORT

Gross Description: The specimen consists of a roughly oval mass of gray-white tissue measuring 5 cm. in length and 2 cm. in thickness. The surface is rough and irregular. Consistence is uniformly firm. The cut section shows a smooth, glistening, gray-white surface with several calcified areas in it.

Microscopic Examination: The groundwork of this specimen is made up of a younger and more undifferentiated cell type than that of the previous case. In places there is a small amount of relatively normal, adult connective tissue with narrow cells, and small vesicular nuclei containing rather little chromatin. The collagen is well developed and runs in wavy bundles of fine fibrils. Fibroglia fibrils are present in considerable numbers. The larger part of the tumor is composed of masses of cells of irregular size and shape, usually oval or polygonal. The nuclei are quite large, and they contain a large amount of dense chromatin. The cytoplasm ranges from slightly acidophilic to strongly basophilic. Single and multiple mitoses are frequently seen. There are many tumor giant cells present with multiple large irregular nuclei containing large masses of chromatin. Foreign body giant cells with rounded nuclei of regular shape and size and a uniform cytoplasm are present as well.

Two types of bone formation may be observed in this tumor. The osteoid type seen in the previous specimen is present but is not the predominating picture. In this specimen the atypical fibroblasts are laying down dense homogeneous intercellular substance which finally becomes true bone. In most places in the tumor the fibroblasts are laying down a blue-staining intercellular substance around each cell. As this material increases, the cells change their shape and appearance to take that of true cartilage cells lying in oval or fusiform cartilage spaces. Bundles of homogeneous fibrils penetrate the cartilage from the adjoining osteoid tissue and this fibrillar material becomes calcified to form true bone. As in the

previous tumor, the bone formation has not the regularity seen in bone of normal growth. There are areas present, however, where the bone formation is relatively normal and of adult type. Rows of osteoblasts are ranged around the edges of the spicules of bone and are laying down more hyaline material. The blood vessels are rather few in number and are extremely well formed. The vascular channels lined by tumor cells described by some observers are not seen. There is no evidence of involvement of muscle.

DISCUSSION

Pathologists have never agreed as to the nature of the cell which forms bone. Kolodny states that the osteogenic sarcoma is a tumor derived from cells which are descendants of mesoblastic elements predestined embryologically to form bone. As evidence of this he states that metastases from osteogenic sarcoma to regions such as the lung, far distant from the primary tumor, show typical bone formation. Mallory, on the other hand, feels that the osteoblast is simply a fibroblast which under certain conditions of stimulation differentiates to form bone. Bone formation in old inflammatory processes is quoted to prove this. Such an osteoblastic function is occasionally taken on by the fibroblasts of the lung in chronic inflammatory conditions. In refutation of this view the point is made that such bone formation is metaplastic rather than neoplastic. It seems to us that the tumors under discussion present all the histologic characteristics which are required to identify an osteogenic sarcoma, although they had no connection with bone and apparently appeared in response to trauma. To make the point clear a discussion is given of the criteria on which the diagnosis of osteogenic sarcoma is made.

The characteristic cell of the osteogenic sarcoma is of the spindle type, varying from small to large size with a hyperchromatic nucleus and cell borders which are difficult to distinguish. These cells are usually considered to be fibroblasts and when proper staining methods are employed, fibroglia fibrils can be demonstrated. Other cells are present which range from spindle to polyhedral in shape and are extremely variable in size. The nuclei are also variable in size and shape and contain a very large amount of chromatin. These cells may take on the usual outline and polyhedral shape of

bone and cartilage cells and are considered to be the characteristic cells of bone tumors. They often show single and multiple mitoses. Tumor giant cells are formed by multiple mitosis of cells of this type and when found are usually considered to be pathognomonic of sarcoma. The formation of hyaline, osteoid, cartilaginous and osseous intercellular substance is seen in both normal and pathological bone formation. Neoplastic ossification is said to differ from metaplastic ossification chiefly in that in the former the fibrillar base for ossification is not preëxisting, but is formed in the process of ossification by way of cell proliferation. Focal proliferation of tumor spindle cells in rows with fibrillation, hyalinization and finally calcification of intercellular substance are the main stages of neoplastic ossification in osteogenic sarcoma. Intracartilaginous ossification is often seen as well. The arrangement of new formed bone is considered to be trabeculated instead of lamellated as seen in physiologically normal bone. There is one criterion accepted by Kolodny and others with which we cannot agree. That is the presence of vascular channels lined by tumor cells instead of endothelium. These structures we have not been able to identify in proved osteogenic sarcomas.

Histologic examination of these two specimens shows that every one of the criteria for the identification of osteogenic sarcoma has been fulfilled by these tumors. All the variations in morphology shown by tumor cells are present. Multiple mitoses and tumor giant cells may be seen. All the varieties of new bone formation are going on and every stage in the process up to adult bone is present.

From these facts we can only conclude that neoplastic bone formation may take place without connection with primitive or adult bone-forming cells. It would appear that under certain conditions fibroblasts can take on the function of tumor as well as of metaplastic bone formation.

Aside from the theoretical considerations these cases are important because they demonstrate that the bad prognosis ordinarily attached to osteogenic sarcoma does not always hold true when a tumor warranting such a diagnosis histologically is found unattached to bone.

These cases illustrate the fact that tumors may develop in subcutaneous tissue not attached to bone which have a histologic appearance identical with that of osteogenic sarcoma. If these speci-

mens had been removed from a tumor which involved bone the prognosis for the patient would have been extremely grave. In the cases reported there has been no evidence of recurrence two years after operation in one case and six months in the other.

SUMMARY

1. Two tumors of soft tissues not attached to bone are described which had a histologic structure identical with osteogenic sarcoma.
2. These tumors have shown no recurrence after local removal.
3. The cases lend support to the theory that the fibroblast in any part of the body may give rise to tumor bone.

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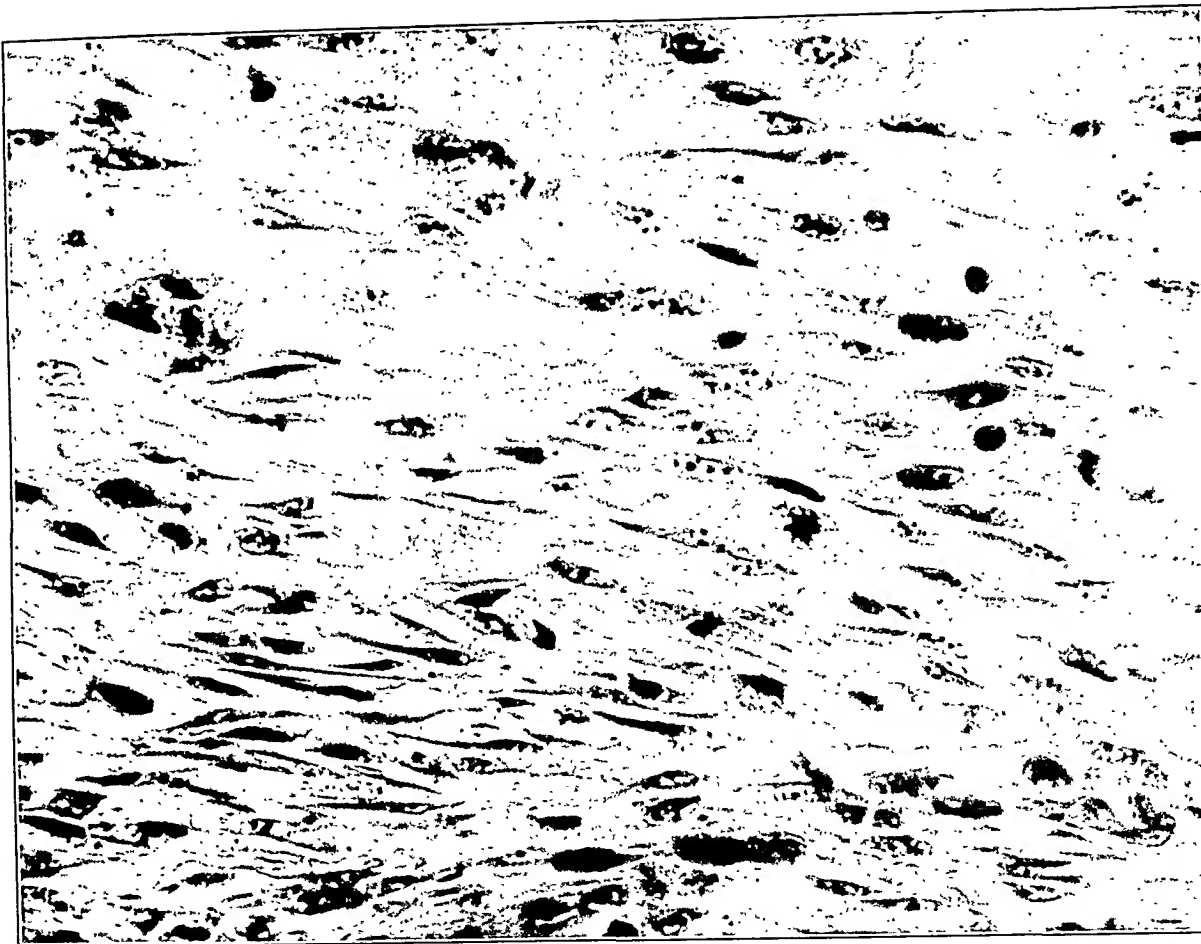
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DESCRIPTION OF PLATES

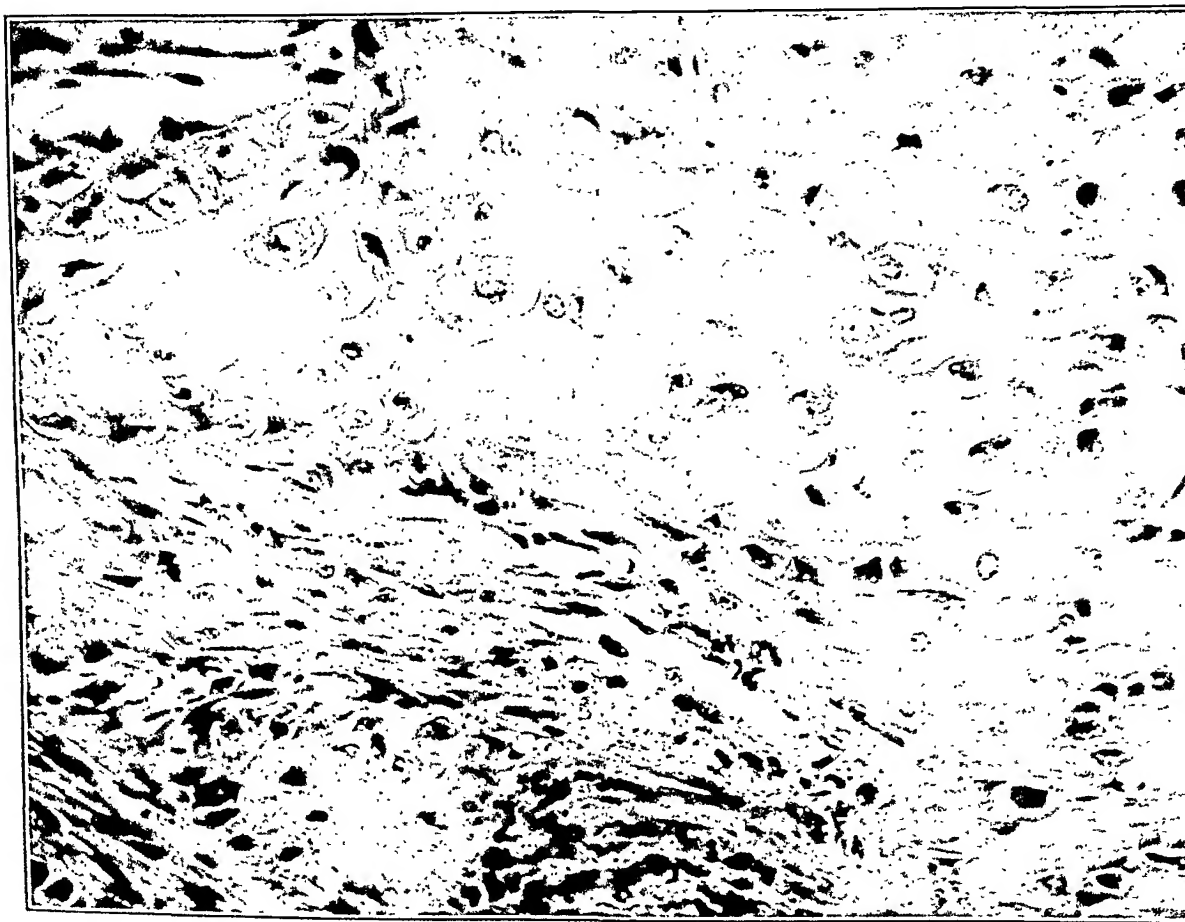
PLATE 83

FIG. 1. A section from Case I showing three mitotic figures. In this region the tumor is growing like a fibrosarcoma. $\times 500$.

FIG. 2. A section from Case I showing cartilage formation and a group of actively growing fibroblasts. $\times 250$.



1

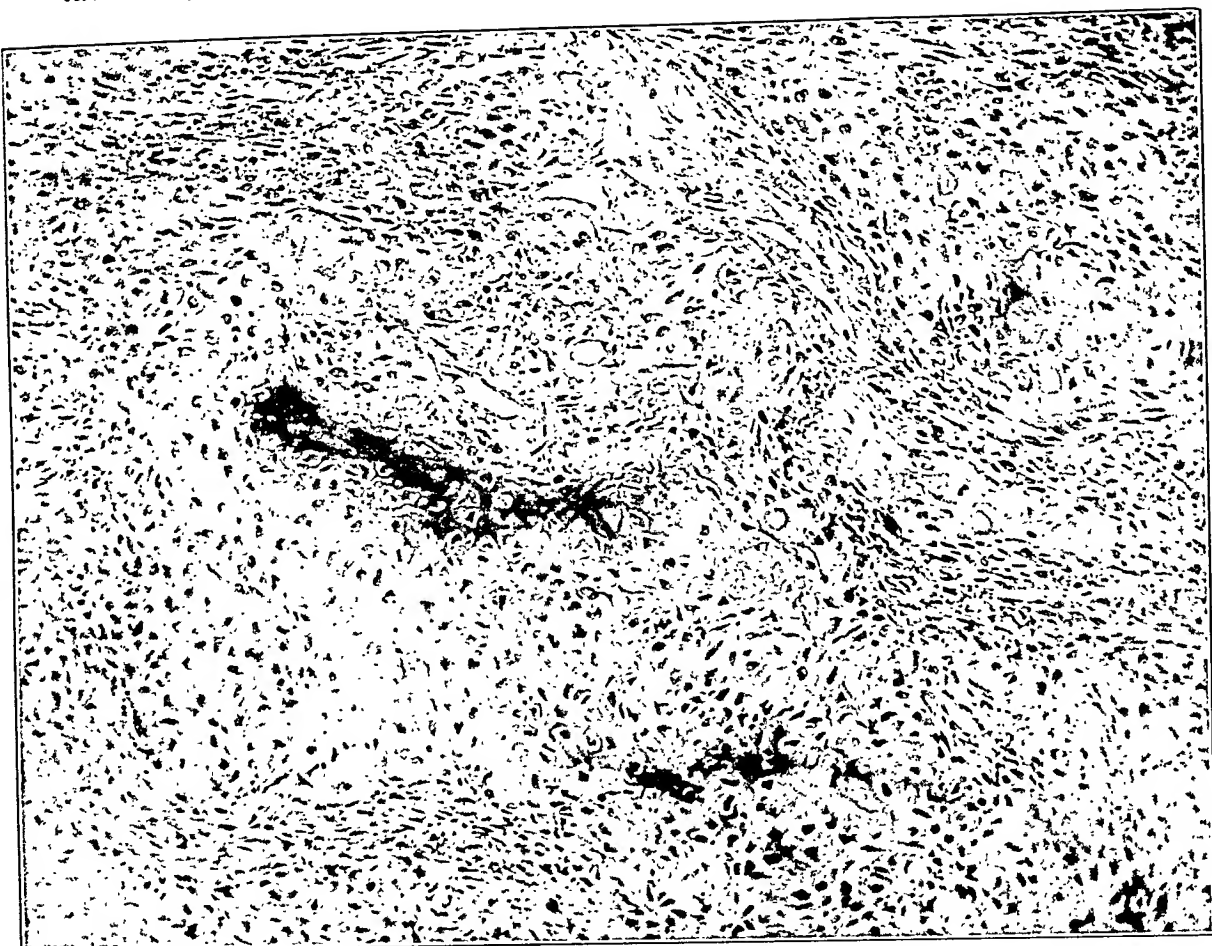


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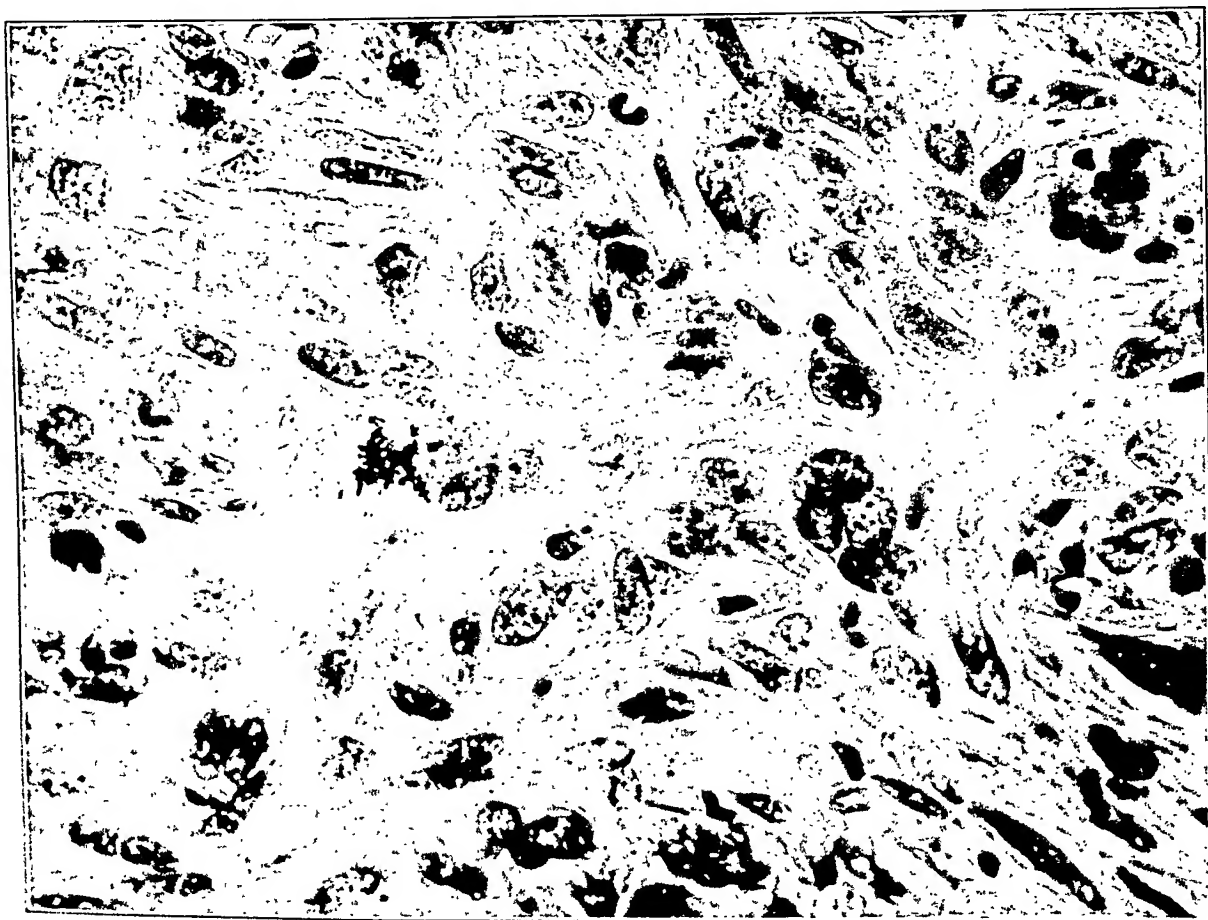
PLATE S₄

FIG. 3. Ossification of intercellular hyaline matrix in Case I. Here bone is being formed directly from osteoid tissue without the presence of cartilage. $\times 100$.

FIG. 4. A rapidly growing area in Case II showing a multiple mitosis and a multinucleated tumor giant cell. Note the irregularity in size and shape of the nuclei. $\times 500$.



3

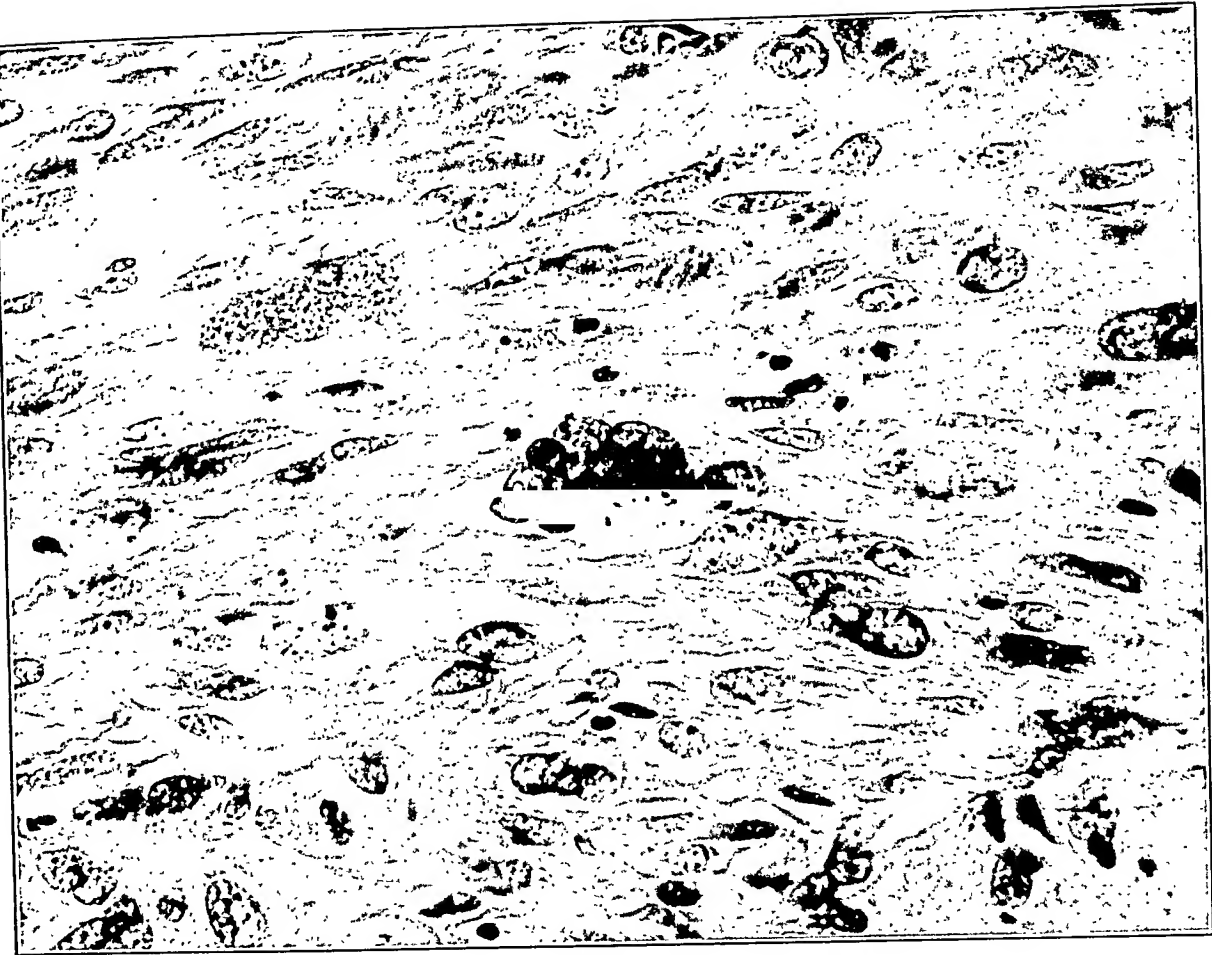


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PLATE 85

FIG. 5. Section from the same case showing a tumor giant cell. Here again the irregularity of cell structure is well shown. $\times 500$.

FIG. 6. Active bone formation in Case II with calcification of spicules of osteoid tissue. $\times 80$.



5



6

A DERMOID OF THE CORNEA IN A GUINEA PIG *

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The purpose of this paper is to place on record a case of dermoid of the cornea observed in an otherwise normal adult female guinea pig. The American Encyclopedia of Ophthalmology¹ states that "primary dermoids of the cornea are extremely rare, most growths involving this structure having their origin in the conjunctiva." Four cases are cited from the literature. In two the tumor was at the limbus, extending over a portion of the cornea and also on to the sclera. In only one of the four cases was histologic study made of the enucleated eye with tumor attached. The latter was the size of a hazel-nut, occupied the entire cornea and encroached upon the sclera. The iris and shrunken, partly calcified lens were adherent to the inner aspect of the cornea.

Fuchs,² De Schweinitz,³ Von Graefe,⁴ Greeff,⁵ and Strawbridge⁶ each report a case, in man, of dermoid at the limbus extending over portions of the cornea and sclera. In spite of the fact that in each instance most of the tumor was over sclera, the respective authors refer to them as corneal dermoids. Greeff mentions two other cases of so-called corneal dermoids in man and one in a dog, that he collected from the literature. Strawbridge quotes Ryba, who collected all cases of dermoid of the cornea published prior to 1853; there were twenty-seven in man, three in oxen, and four in dogs. The bilateral occurrence of dermoids extending over portions of cornea and sclera is reported by Taliaferro⁷ and Virchow.⁸ Noyes⁹ describes three sessile dermoid tumors in the eye of a man "growing upon the limbus corneae equidistant from each other."

GROSS DESCRIPTION

In the center of the cornea of the guinea pig's right eye is a yellowish, fleshy, round, convex disc bearing a tuft of hair. The diameter of this disc is 5 mm., and in its center at the point of greatest convexity it is 3 mm. thick. There are no dermal or vascular connec-

* Received for publication May 10, 1928.

tions visible in the gross between the disc and the palpebrae. The sharp line of transition between transparent cornea and opaque disc gives to the latter the appearance of being "stuck on." The tuft of hair is gray, the same color as the fur of the rest of the body. But, whereas the latter is smooth (lying down) the hairs of the tuft stand out perpendicularly to the surface of the disc.

The right eye exhibits no other abnormalities. The distance between the canthi is 1.2 cm. in each eye, but the right eye appears slightly more prominent than the left.

MICROSCOPIC STUDY

Methods: The guinea pig was killed by etherization. The head was separated from the body and fixed in Kaiserling's solutions. The right eye was dissected out with palpebrae attached, no other anomalies being noted. It was then embedded in celloidin in the usual manner and sections approximately 20 microns in thickness were cut for study. The stains used were hematoxylin and eosin.

Microscopic Findings: The cross-section of the tumor is roughly a half-circle, convex surface externally. It is composed mainly of fat tissue and rests directly upon substantia propria corneae. It is covered with stratified squamous epithelium whose basal layer contains pigment, and beneath which is a layer composed of interlacing bundles of fibroblasts containing sebaceous glands and arrectores pilorum muscles. This layer corresponds to the corium of the normal integument. Passing through this layer or corium are hairs whose bulbs are embedded in the areolar tissue beneath. In the corium and fat tissue are capillaries, venules and small arterioles.

On each side of the tumor, cornea and conjunctiva are normal. At the margin of the tumor, the layer of stratified squamous epithelium that covers it becomes continuous with the conjunctiva. In this region also the substantia propria corneae splits into two portions. An external, smaller portion becomes continuous with the layer of the corium of the tumor, and the larger inner portion continues under the tumor forming the base upon which it rests. The anterior basal lamina of the cornea is clearly defined in the normal portions but may be followed only a short distance over the edge of the tumor. Here it rapidly loses its identity, becoming continuous with that part of the corium immediately beneath the layer of

stratified squamous epithelium. In the marginal regions of the tumor are also anastomoses between capillaries of the corium and of the conjunctiva. A large venule and an arteriole are seen passing between the corium of the tumor and the subconjunctival tissue.

DISCUSSION

Concerning the origin of corneal dermoids nothing can be added to what has been already said in regard to the origin of dermoids in general. The occurrence of true corneal dermoids, as previously mentioned, is very rare, and in the opinion of the writer still less frequent than the literature would indicate. For, as pointed out, not only were a number of dermoids, reported as corneal in origin, situated at the limbus with more of the tumor over sclera than cornea, but microscopic study of them *in situ* was not made. Their classification, therefore, as true corneal dermoids is not justified. Noyes⁹ and Alt¹⁰ regard all dermoids at the corneo-scleral junction as conjunctival in origin. This view is probably correct.

The following evidence indicates that the dermoid described in this paper is corneal and not conjunctival in origin:

1. The entire base of the tumor rests upon substantia propria corneae, nothing intervening.

2. The substantia propria corneae splits at the margin of the tumor, one part becoming continuous with a part of its superficial wall, the other passing beneath it.

3. As a corollary to the preceding observation, the layer of substantia propria corneae beneath the tumor is narrower than it is at the sides of the tumor (*i.e.*, before it splits).

4. The anterior basal lamina of the cornea extends into the tumor becoming continuous with the most superficial portion of the layer of corium.

The foregoing facts are irreconcilable with a conjunctival origin. If this were the case a dermoid of this size would rest upon the cornea, not in it. The cornea would not split at the margins of the tumor to include it, as it were. The anterior basal lamina would pass beneath the tumor and not become continuous with its most superficial part. Or if there was secondary invasion of the cornea, the anterior basal lamina would be traced into the basal regions of the tumor.

SUMMARY

1. A case of true dermoid of the cornea in a guinea pig is recorded.
2. All dermoids reported as corneal in origin cannot be finally classified as such because of lack of microscopic evidence and because many of them were at the limbus with more of the tumor extending over sclera than cornea.

The author wishes to thank Miss Lillian M. Leavitt for the preparation of the sections and Dr. F. B. Mallory and Miss Catherine G. Norton for the illustrations.

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 DESCRIPTION OF PLATES

PLATE 86

FIG. 1. Guinea pig showing erect position of hairs of right corneal tumor.

FIG. 2. Head of guinea pig after separation from body and fixation in Kaiserling's solutions. The eye has been rotated superiorly and anteriorly to show lateral view of the corneal tumor. Note "stuck on" appearance of tumor. Some of the hairs have dropped off.



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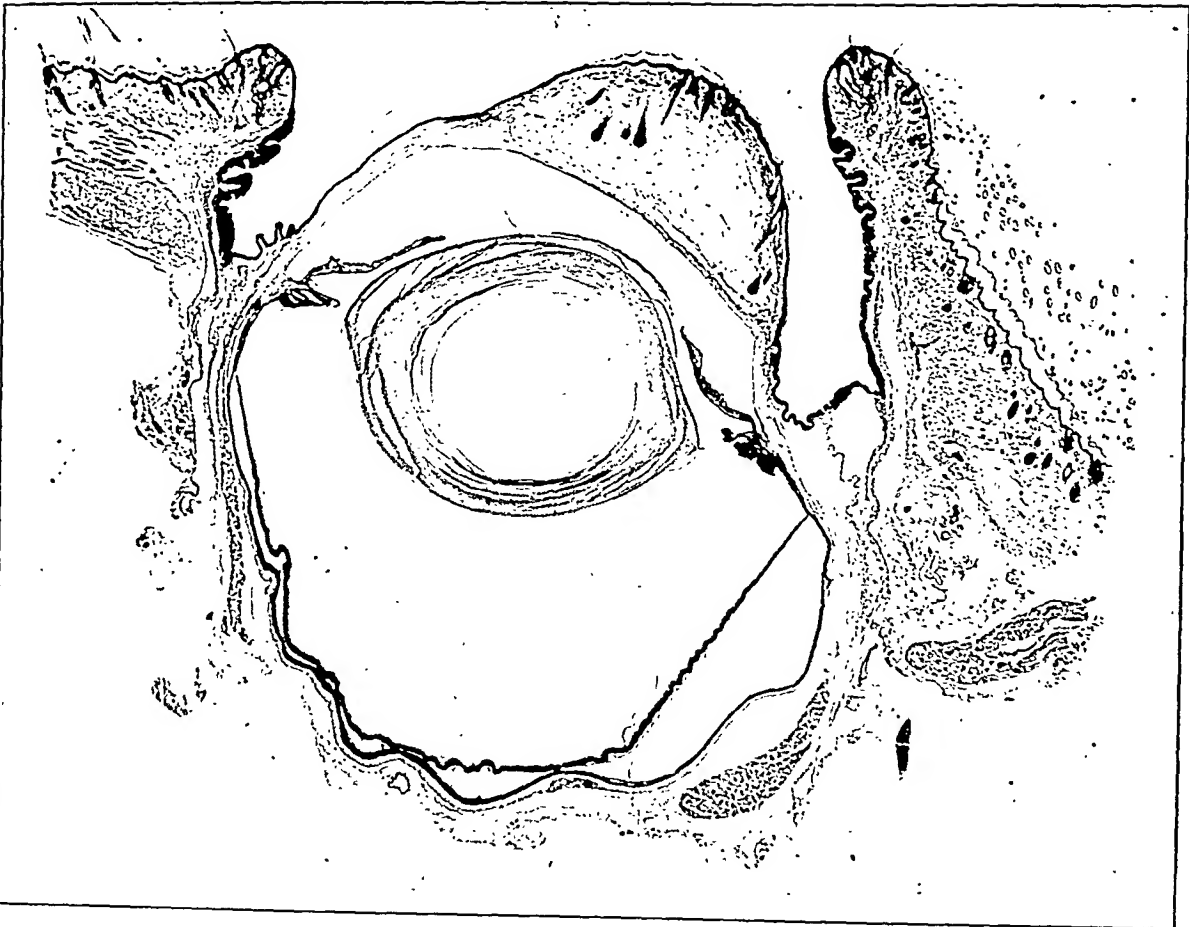


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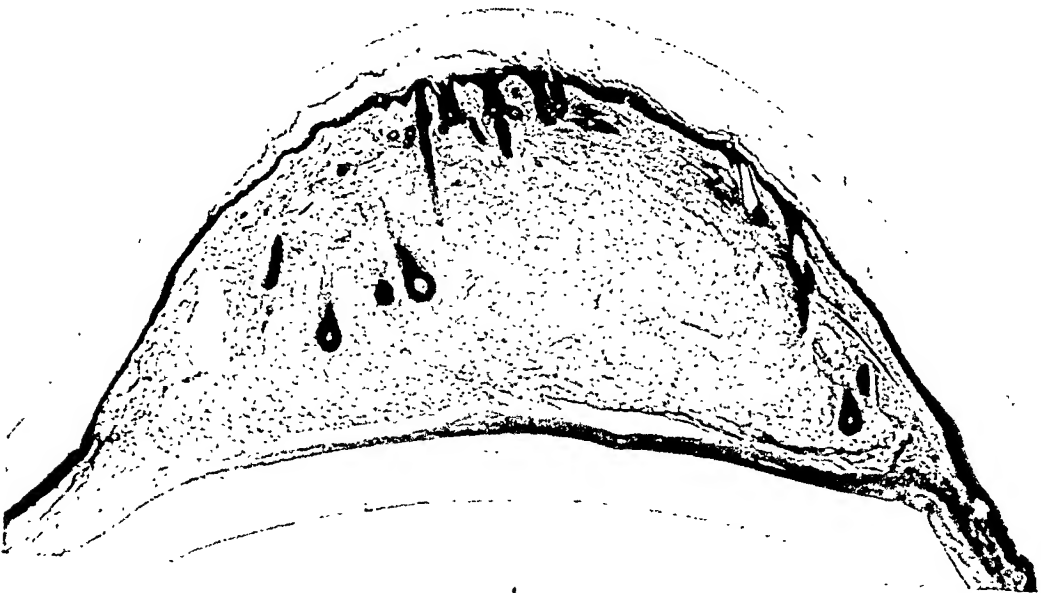
PLATE 87

FIG. 3. Sagittal section through eye, tumor, and palpebrae, approximately in midline. Some distortion due to fixation. During life tumor was in center of cornea. Palpebrae, lens, ciliary body, wall of bulbous oculi are normal. $\times 8$.

FIG. 4. Enlargement of tumor seen in Fig. 3. Sagittal section through midline of tumor, which is composed mainly of areolar tissue, covered externally by stratified squamous epithelium. Beneath the latter is layer corresponding to corium of normal integument, and containing sebaceous glands and arrectores pilorum muscles. Bulbs of hair in areolar issue. Hair shafts passing through corium. Tumor rests upon substantia propria corneae. At margin of tumor stratified squamous epithelium becomes continuous with conjunctiva. Substantia propria corneae splits into two portions. The smaller external portion is continuous with layer of corium, the internal larger portion passes beneath tumor. $\times 15$.



3



4

OBSERVATIONS ON INCUBATED TISSUES AND EXUDATES *

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In two recently published articles the authors have described certain cells which develop in incubated leukemic and normal blood.^{1, 2} The appearance and activity of these cells under various abnormal conditions were discussed in a third paper.³ The cells which were seen in these experiments were so striking and characteristic in their appearance that we thought it a matter of interest to determine whether or not the same types of cells would develop in tissues kept under similar conditions. We felt that from a study of tissues it might be possible to determine what cells are the progenitors of the large cells of the blood.

In the previous papers we have defined the various terms used. It is sufficient to say here that we follow essentially the nomenclature used by Sabin, Doan and Cunningham.⁴

LITERATURE

Although a large amount of work has been done on tissue cultures little attention has been paid to the classification and description of the wandering phagocytic cells which develop in them.

Lewis, Willis and Lewis⁵ used plasma clot, coverslip preparations of tuberculous tissue and watched the development of epithelioid cells. They thought that such cells arose from either monocytes or clasmatocytes and felt that they could see transition stages between both these types and epithelioid cells.

Lewis and Webster^{6, 7} described experiments in which they cultured tissue from normal, and acutely and chronically inflamed lymph nodes. Nodes of Hodgkin's disease, tuberculosis, carcinoma, melanotic sarcoma, and lymphatic and myelogenous leukemia were also used. Giant cells developed in all of these cultures, which when stained supravitaly showed a central red zone and peripherally arranged fat globules. They described the appearance of a wander-

* Received for publication April 14, 1928.

ing cell which migrated out of the tissue during the first few hours of incubation, kept moving for forty-eight hours and then became quiescent and died. A second type was seen which was called the endothelial type and which contained a well marked rosette stained with neutral red. All transitions could be seen between wandering cells and endothelial cells, and between endothelial cells and giant cells. The endothelial cells did not begin to migrate from the tissue until after twenty-four to forty-eight hours of incubation. They were cells of large size, irregular outline, and rather sluggish motility. Forms simulating wandering cells and fibroblasts were seen. These workers never saw transition stages between lymphocytes and the wandering or endothelial types of cell.

Maximow⁸ cultivated rabbit mesenteric lymph nodes, omentum and loose connective tissue. The preparations were made in plasma clots on coverslips, and bone marrow extract was used to supply the required growth-stimulating substance. The cultures were inoculated with human tubercle bacilli in order to watch the development of tubercles *in vitro*. The large, pale cells of the lymph nodes, including those lining lymph sinuses, were seen to proliferate and become ameboid and phagocytic, sometimes epithelioid in type. They stored dye and formed giant cells. Lymphocytes were also seen to change in appearance, enlarge, proliferate and to become ameboid and phagocytic. Maximow believes that a large group of primitive cells, mesenchymal in type, exist such as the clasmatocytes of the tissues, the reticular cells and cells lining the sinuses of lymph nodes, spleen and bone marrow, and the Kupffer cells of the liver sinusoids. He holds that these cells are pluripotential and that when stimulated by the use of various dyes and colloidal materials they may become actively phagocytic. He feels that under other conditions they may give rise to lymphocytes and even to granulocytes.

Carrel and Ebeling,^{9, 10, 11} studied a long series of cultures in which both tissue and blood cells were examined. They found that cultures in serum allowed the growth of mononuclear cells only, whereas in plasma clots both fibroblasts and mononuclear cells grew out. They describe the transformation of monocytes into clasmatocytes and also into fibroblasts.

McJunkin¹² described observations on lymph nodes and on peritoneal exudates of rabbits, although no cultures were made. He saw both "rosette" and "non-rosette" cells in normal rabbit lymph

nodes. By "rosette cell" he meant a cell with a considerable clump of fine red granules in the indentation of the nucleus. He stated that all transitions were present from the "reticular" cells with a single small collection of fine granules to those with rosettes and numerous scattered red granules. From his experiments he concluded that the blood monocyte arose in lymph nodes and that the larger cells with irregular neutral red bodies were derived in part from the blood monocytes and in part from vascular endothelium.

Sabin holds that no monocytes are found in rabbit nodes and that large numbers are present in the spleen. She considers that they arise from a primitive reticular cell which exists in spleen, liver, lung and the milk spots of the omentum. The clasmatoocyte according to her theory arises from fixed endothelium of the type found lining the sinusoids of the liver or the lymph channels of the spleen and lymph nodes. She feels that the monocytes and clasmatoocytes are quite distinct types and that one never becomes the other.

Sugiyama¹³ and other embryologists state that while cells of the clasmatoocyte type may be seen in chick embryos after the first few days of incubation, monocytes do not appear until about the second week. This point is of great importance in proving that the two types of cells are of a different origin.

CULTURES OF EMBRYONIC TISSUE

In our experiments, a series of chick embryos of various ages up to ten days were cut in small pieces and suspended in tubes of serum. They were incubated at 37.5° C and control tubes were kept in the icebox. Bits of tissue were removed with a platinum loop and mounted on neutral red slides for supravital observations. The only motile phagocytic cells seen before incubation were clasmatoocytes.

At the end of twenty-four hours a fair number of cells had appeared. Most were of the clasmatoocyte type and contained many neutral red granules of irregular size and shape. The cell outline was often indented or fusiform. A few cells were seen which showed a rosette arrangement of fine red granules in the center of the cell. Outside this was a row of regular refractile granules. These cells were less actively motile and showed a more uniform outline than those of the clasmatoocyte type. They were considered to be monocytes. Cells of these same types persisted for several days until the death of the cultures.

Rabbit embryos, eighteen days old, were used in a somewhat similar experiment. Both coverslip, plasma clot preparations and bits of tissue suspended in serum were set up. Embryonic liver and subcutaneous tissue were used. No monocytes were noted in spreads of the tissues made before incubation. At the end of twenty-four hours a number of cells were seen which had a fairly regular outline, a well marked focus of fine neutral red granules, an indented nucleus and a peripherally arranged row of refractile granules. A second type of cell was found which had a more irregular outline, a large number of red granules of various sizes and depth of color and rather few refractile granules. At the end of forty-eight hours incubation the same types of cells were present and were considerably larger than before. At the end of three days the refractile granules were very marked in the rosette type. The rosette was of good size and composed of rather finely divided masses of neutral red. The second type showed far fewer refractile granules and more variable masses of neutral red though a rosette of fine granules was often present. The cells were very irregular in shape and many elongated forms were seen. At the end of four days incubation the picture was essentially the same and after six days the cells were all dead.

From these experiments we may conclude that clot and serum cultures of chick and rabbit embryos show the same changes in the wandering cells as are seen in incubated adult human and rabbit blood. Rosette and non-rosette cells develop, hypertrophy and show refractile granules, more in the rosette than in the non-rosette type.

EXUDATES

The formation of exudates in the pleural cavities of guinea pigs was induced by the injection of 5 cc. of ordinary bacteriologic beef infusion broth containing 0.1 per cent of dextrose. The exudate was withdrawn after twenty-four hours and examined supravitaly. Two types of cells could be seen besides lymphocytes and granulocytes. One was of large size and irregular outline. The cytoplasm was packed with variously sized masses of neutral red. Phagocytosed erythrocytes and polymorphonuclear cells were seen. The second type was smaller and tended to be round. A focus of fine neutral red granules was present near the nucleus. The cytoplasm contained a large number of refractile granules arranged peripherally. Only a moderate amount of phagocytosis was seen in this type of cell.

Exudates were produced in rabbits by the intrapleural injection of 5 cc. of the animal's own blood removed by cardiac puncture and injected before the blood clotted. Specimens were withdrawn at various periods and observed in supravital spreads and in fixed preparations. After twenty-four hours the cells were almost identical with those seen in the tubes of blood after several days incubation. It was possible to identify two types of cells. One showed a definite, rounded focus of fine neutral red granules surrounded by a peripheral zone of unstained refractile granules. This cell was usually from ten to forty microns in diameter and had quite a regular outline. There was some phagocytosis of red cells. The second type was a larger cell of more irregular shape which tended to be more actively motile. This type of cell had a cytoplasm almost filled with red-staining masses of phagocytosed material. In the dried preparations stained by Wright's method the first type of cell showed a round to oval nucleus with rather light staining chromatin. The cytoplasm was often entirely filled with unstained vacuoles. The second type of cell had one or more round to slightly oval nuclei containing fairly dense chromatin. The cytoplasm was a fairly uniform blue in color and contained irregular masses of red-staining material. Sometimes a few basophilic granules were present.

Ascitic fluid was removed from a patient who had been ill with chronic lymphatic leukemia for several years. Fixed and supravital preparations of the fluid showed it to contain a large number of typical small lymphocytes, none showing more than one to two granules of neutral red. No monocytes were seen in counting four hundred cells. Tubes of this fluid were incubated at 37.5° C and observations were made every two days. On the sixth day cells appeared which were many times the size of a lymphocyte. They had an indented nucleus often containing one or more nucleoli. A few refractile granules were present. Rather fine and diffusely scattered neutral red granules were seen, usually collected in a focus in the cytoplasm. This type of cell was extremely phagocytic, often containing large masses of red-staining material or carbon in the cytoplasm. In the Wright's stained preparations a large number of typical "X" cells were found. The nucleus was oval or sometimes indented. Many multinucleated forms were present. There was a large amount of evenly stained blue cytoplasm which often contained masses of red-staining material or small basophilic granules.

Phagocytosed carbon granules were often present. The oxidase reaction was both positive and negative. These cells remained alive for several days and then all died.

The results of this experiment seem to bear out the work of Bloom.¹⁴ This investigator made cultures of clotted lymph obtained from the thoracic duct. The lymphocytes originally present proliferated, hypertrophied and became ameboid and phagocytic. Observed in supravital spreads these cells were quite typical monocytes with well formed rosettes. From these results he argued that monocytes took their origin from lymphocytes and were simply one type of the polyblast of Maximow.

ANIMAL TISSUES

A series of cultures of rabbit lung, spleen, liver, lymph node, bone marrow and subcutaneous tissue were examined. The tissue was cut into small pieces and several bits placed in test tubes containing about 0.5 cc. of homologous serum. Supravital and stained preparations were made at frequent intervals and the changes in the cells observed. Before incubation cells of both the clasmatocyte and monocyte type were present in all tissues examined except the lymph nodes. Here only clasmatocytes were seen.

At the end of twenty-four hours incubation only a few cells had appeared. These were of both the rosette and non-rosette type. The size was about that of the ordinary monocyte and no refractile granules were present.

After forty-eight hours all the cultures showed a good growth of cells and two types were seen. One had a well marked rosette, often of the hypertrophied type, composed of many fine red granules, little phagocytosed material and few refractile granules. The second type contained a smaller focus of neutral red granules close to the nucleus and a large number of refractile granules.

As the period of incubation went on the first type of cell containing the large rosette of fine granules enlarged greatly and became very actively phagocytic. The second type did not increase so much in size, preserved a definite, though often very small rosette, and exhibited a large number of refractile granules in the cytoplasm.

A similar series of observations were made on a group of animals which had received repeated injections of streptococci. These ani-

mals showed a very high count of monocytes in the blood. The organs such as the lung, spleen, liver and bone marrow were found to contain a large number of these cells with hypertrophied rosettes. Cultures of these organs showed a large number of cells with variably sized neutral red rosettes and many refractile granules. Frequently these cells grew to tremendous size but preserved the same general appearance. A very limited number of cells with irregular outline, ill-defined rosettes and active phagocytosis were seen.

From this experiment it is possible to conclude that the monocyte is the progenitor of the rosette, refractile granule type of cell which we have called a "Y" cell in previous studies.

HUMAN LYMPH NODES

A series of human lymph nodes were examined by a technique similar to that used with the animal tissues. The specimens also were all run through the routine histologic technique of paraffin embedding, sectioning and staining with eosin-methylene blue. Several showed only chronic inflammation, one contained metastatic fibrosarcoma and two were from typical cases of Hodgkin's disease. The tissue was brought aseptically from the operation, cut in small pieces and suspended in either sheep serum or ascitic fluid. It was known from previous observations that both of these fluids made good media for the development of phagocytic cells. Supravital and fixed observations were made on bits of tissue before incubation. After the preparations were set up pieces of tissue were fished out of the medium with a platinum loop and observations were made on both supravital spreads and on smears stained by Wright's method and by the oxidase reaction. To make the discussion of the findings clear it is necessary to define the terms used.

"Reticular" cell refers to a group of cells as large or larger than a blood monocyte. The nucleus contains a rather small amount of chromatin and is of good size, sometimes indented and often lobed. The amount of cytoplasm is often variable. Some of the cells have little more cytoplasm than a large lymphocyte and others have quite a large amount. There is a focus of rather fine, uniform, neutral red granules closely related to the indentation of the nucleus and often occupying nearly half of the total cell cytoplasm. Unstained refractile granules are very few.

Lymphoblast refers to a cell from ten to fourteen microns in diameter. The nucleus is round, of good size, and has a peculiar ground glass appearance when examined vitally. There is rather little cytoplasm as compared with the size of the nucleus and few or no neutral red granules are seen.

The "X" cell is a cell varying in size from that of a large lymphocyte up to a form somewhat larger than the reticular cell and has a rather irregular outline. The nucleus is round or oval and more than one nucleus may be present. The cytoplasm is variable in amount and contains a large number of fine, uniformly sized neutral red granules scattered irregularly throughout. Phagocytosis may be quite active in this type of cell and sometimes the cytoplasm is almost filled with engulfed material. In fixed preparations the nuclei are usually rounded and contain a moderate amount of chromatin. There is a large amount of even blue cytoplasm often containing red-staining masses of phagocytosed cellular material, carbon or fine basophilic granules.

The "Y" cell, as previously described, is a cell seen in incubated blood, which has a round or oval nucleus, a group or a small rosette of rather coarse neutral red particles close to the nucleus and the cytoplasm filled with refractile granules. Phagocytosis is not marked and the cell outline is round and uniform.

Before incubation all the specimens showed four types of cells.

1. Small lymphocytes. These cells had round nucleus and a small amount of cytoplasm containing a few small granules of neutral red.

2. Cells varying in size from slightly larger than a small lymphocyte to a cell twenty or thirty microns in diameter. The nuclei varied from round to oval or indented. In the cytoplasm in close relation to the nucleus there was a rounded focus of fine neutral red granules often similar to the hypertrophied rosette described by other workers.

3. A variable number of cells morphologically similar to human blood monocytes. These cells were from ten to fourteen microns in diameter, contained an indented nucleus and a small unstained area near the indentation around which a few neutral red granules were grouped.

4. Fibroblasts and fixed endothelial cells were seen which contained scattered fine red granules at either end of the nucleus.

The nodes of Hodgkin's disease contained cells unlike those seen in any other condition. These cells ranged from twenty to forty microns in diameter and were rounded and regular in outline. The nucleus was round and very large, occupying a good part of the cell. In the nucleus were one or more large round nucleoli. These cells took no neutral red.

After twenty-four to forty-eight hours incubation the cultures showed a very striking picture. Examined in supravital spreads great masses of cells of one type were seen which varied in their size and content of neutral red granules. Every conceivable stage of rosette formation was present from a few red granules to a mass of fine red particles which filled a large part of the cytoplasm. There were a number of unstained refractile granules. Stained by Wright's method they tended to have an oval or indented nucleus and a variable amount of blue cytoplasm. These were considered to be reticular cells.

A second type of cell was present which was somewhat larger and more irregular in outline than the cell just described. This type showed a large number of irregular red particles scattered without arrangement through the cytoplasm. The outline of the cell was likely to be stellate or elongated. Many of this type were motile. There was often a considerable amount of phagocytosed material in the cytoplasm. This type of cell is identical with the "X" cells seen in incubated blood.

Fair numbers of small lymphocytes were scattered among the large cells. These tended to have more red granules in the cytoplasm than the cells of that type seen in the fresh preparations.

The oxidase stain on smears of tissue before incubation showed in the Hodgkin's cases a few oxidase-positive mononuclear cells in the fixed preparations, which resembled blood monocytes very closely. In the nodes of Hodgkin's disease a very large number of cells were oxidase-positive at the end of forty-eight hours incubation. From that time on the number of oxidase-positive cells decreased until few or none were seen at the sixth or eighth day of incubation.

Two main points of difference were seen between cultures of Hodgkin's disease lymph nodes and the simply inflammatory nodes. One was the presence of the large cell previously described in the Hodgkin's disease cases. The second was the occurrence of many oxidase-positive as well as negative "X" type cells in Hodgkin's

while the "X" cells in preparations from inflammatory nodes were all oxidase-negative.

From the second day of incubation on, the only changes in the cultures were the gradual enlargement of the reticular cells, the appearance of more "X" and "Y" type cells, and the disappearance of the oxidase-positive cells in the Hodgkin's disease cases. The average length of life of the cells was about six days but in a few instances they lived as long as ten or twelve days. As the cultures became old the cells lost their characteristic morphology and became filled with large, irregular masses of hemoglobin.

As previously described, two types of cells which we have called "X" and "Y" develop in incubated blood. In observations on lymph nodes similarly incubated cells of exactly the same morphology, staining properties and physiologic activities as those seen in incubated blood have been observed. In addition a third type of cell appears in the lymph node cultures which we have called a "reticular" cell. The oxidase reaction of this cell may be either positive or negative. Every stage in the transition between "reticular" cells and "X" cells may be seen. Monocytes similar to those seen in the blood are found in certain cases, and "Y" type cells, which we suppose come from monocytes, develop in these cultures.

SUMMARY AND DISCUSSION

Tissues from chick and rabbit embryos of various ages were incubated in rabbit serum. Observations made on the tissues before incubation showed only clasmatoocytes and no monocytes. After twenty-four hours incubation, cells of the monocyte type with definite neutral red rosettes appeared. This observation would argue that some predecessor of the monocyte is present in early embryonic tissue. There is enough similarity between the wandering phagocytic cells of the clasmatoocyte type and the monocytes to make it conceivable that one gives rise to the other.

Pleural exudates induced in rabbits and guinea pigs were examined. Cells were seen which were almost identical with the cells appearing in incubated blood. This is some proof that the cells seen in the blood cultures were not degeneration forms. If the cells of the exudates were suspended in serum they would remain alive for considerable periods.

Ascitic fluid from a case of chronic lymphatic leukemia was found to be almost a pure suspension of cells of the type of small lymphocytes. This fluid was incubated and about the sixth day a large number of good sized, actively phagocytic cells, sometimes multinucleated, appeared which were very much like the "X" type of cell found in incubated blood. Unfortunately, part of the tubes were spoiled and repeated observations to watch transition stages could not be made. This experiment corresponds to the work of Bloom, who cultivated large phagocytic cells from the lymph of the thoracic duct. Such lymph has been shown to contain only lymphocytes.

Pieces of spleen, bone marrow, lung, lymph node and subcutaneous tissue of rabbits were examined supravivally and then incubated in sheep serum. Cells of both clasmatocyte and monocyte types were present in all the tissues examined before incubation except the lymph nodes where only clasmatocytes were seen. After incubation, however, large cells appeared in all the tissues, which were identical with those seen in incubated blood. In tissues from animals in which a monocytosis had been induced before death a larger number of cells of the rosette type with refractile granules were seen in the cultures.

The most interesting experiments were those on human lymph nodes. These are of particular importance in view of the observations of Sabin that monocytes are not present in normal nodes and of McJunkin that the monocyte is the cell involved in Hodgkin's disease. The incubated specimens showed all transitions between cells about the size of a lymphocyte which contained a small number of neutral red granules grouped in a rosette up to very large cells with one or more nuclei and a rounded mass of neutral red granules identical in morphology with the hypertrophied rosette seen in monocytes in tuberculosis. The number of these cells which appeared during incubation was apparently directly proportional to the amount of inflammatory reaction present in the node to begin with and had no relation to the amount or type of tumor present. The cells were phagocytic, often motile, and contained a variable number of refractile granules. In the nodes of Hodgkin's disease many of these cells were oxidase-positive for a period.

We are indebted to Dr. F. B. Mallory and Miss Catherine Norton for the photomicrographs.

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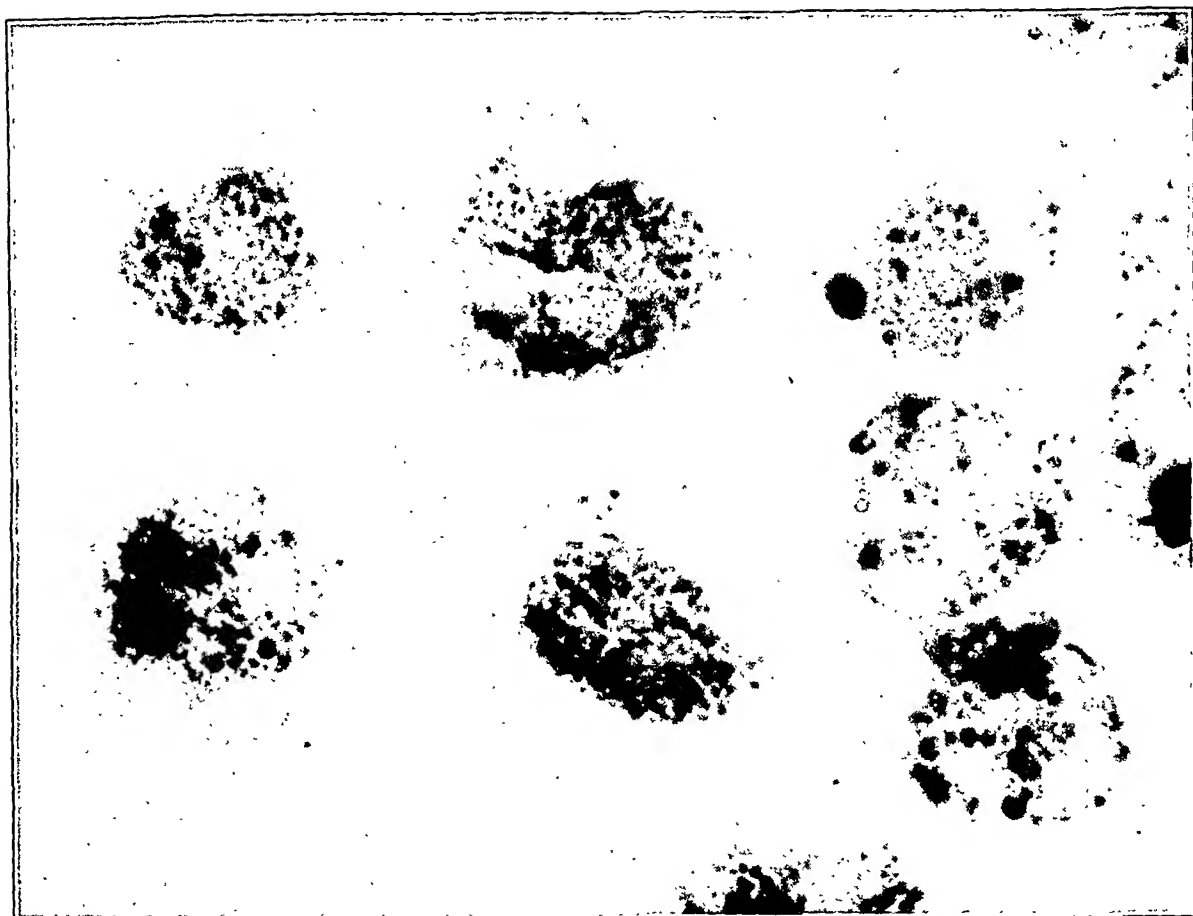
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DESCRIPTION OF PLATES

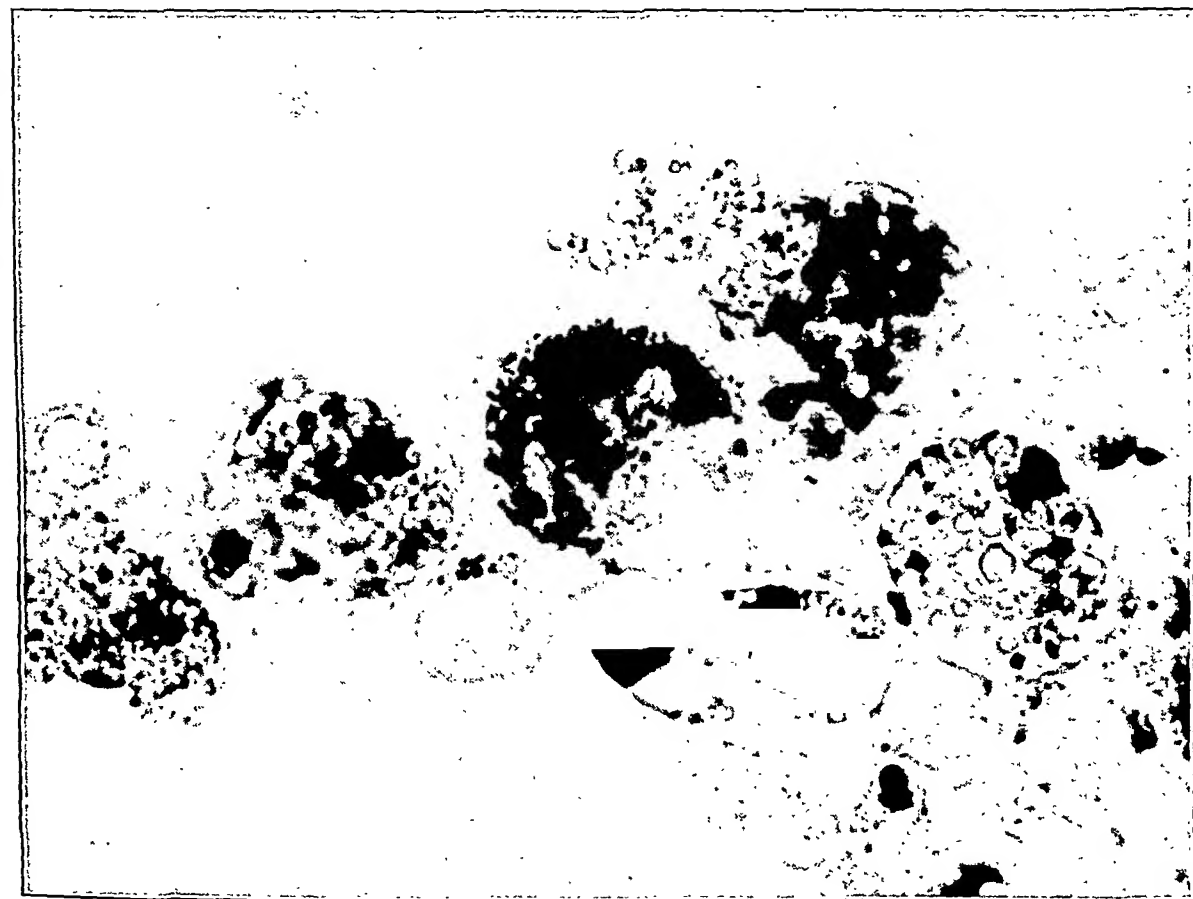
PLATE 88

FIG. 1. A group of "reticular" cells from a chronic inflammatory lymph node incubated seven days. There are five typical "reticular" cells with well marked deeply stained rosettes. The cell in the upper right corner in addition shows a phagocytosed particle at the periphery of the rosette. The rosettes as can be seen are composed of many fine granules of approximately equal size and occur in the hofs of the indented nuclei. The two entire cells in the lower left corner are not characteristic enough to classify. A supravital preparation. $\times 2000$.

FIG. 2. A clump of cells from a Hodgkin's disease lymph node, incubated seven days. Three of the cells are "Y" cells. The neutral red in them occurs in granules and globules of unequal size and with an uneven distribution. Note the large unstained refractile granules characteristic of this type of cell. The small cell near the center is a lymphocyte showing a few fine neutral red granules. Above it to the right is an actively phagocytic "X" cell with a few fine unstained refractile granules. The clear portion of the cytoplasm at the periphery of the rosette can be seen. The elongated cell below this is another "X" cell containing masses of darkly stained hemoglobin. A supravital preparation. $\times 2000$.



1



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PLATE 89

- FIG. 3. Cells from a chronic inflammatory lymph node incubated five days. The second cell from the left at the bottom with the large round nucleus and prominent nucleoli is a young "reticular" cell; it shows a small rosette at one side of the nucleus. Above it, to both the left and right are older "reticular" cells with indented nuclei and well defined rosettes. The remainder of the cells are typical small lymphocytes. A supravital preparation. $\times 2000$.
- FIG. 4. An "X" cell from an incubated chronic inflammatory lymph node. It contains many large neutral red granules and globules around its poorly defined rosette. The border is irregular due to the numerous pseudopods. A supravital preparation. $\times 1000$.
- FIG. 5. A clump of cells from a lymphoblastoma lymph node incubated two days. The cell at the center is an "X" with large amount of cytoplasm and eccentric nucleus. Several of the other cells have a considerable amount of cytoplasm and are irregular in outline but are smaller than the "X" cell; these are "reticular" cells. In addition there are a few lymphocytes. The two vacuolated structures are nuclei of dead cells. Wright's stain. $\times 1000$.
- FIG. 6. A binucleated "X" cell from ascitic fluid of a case of chronic lymphatic leukemia, after six days incubation. There are several phagocytosed particles in the cytoplasm. The cytoplasm also shows a considerable number of fine granules and a darker area to the left of the nuclei suggesting the rosette area. Wright's stain. $\times 1000$.



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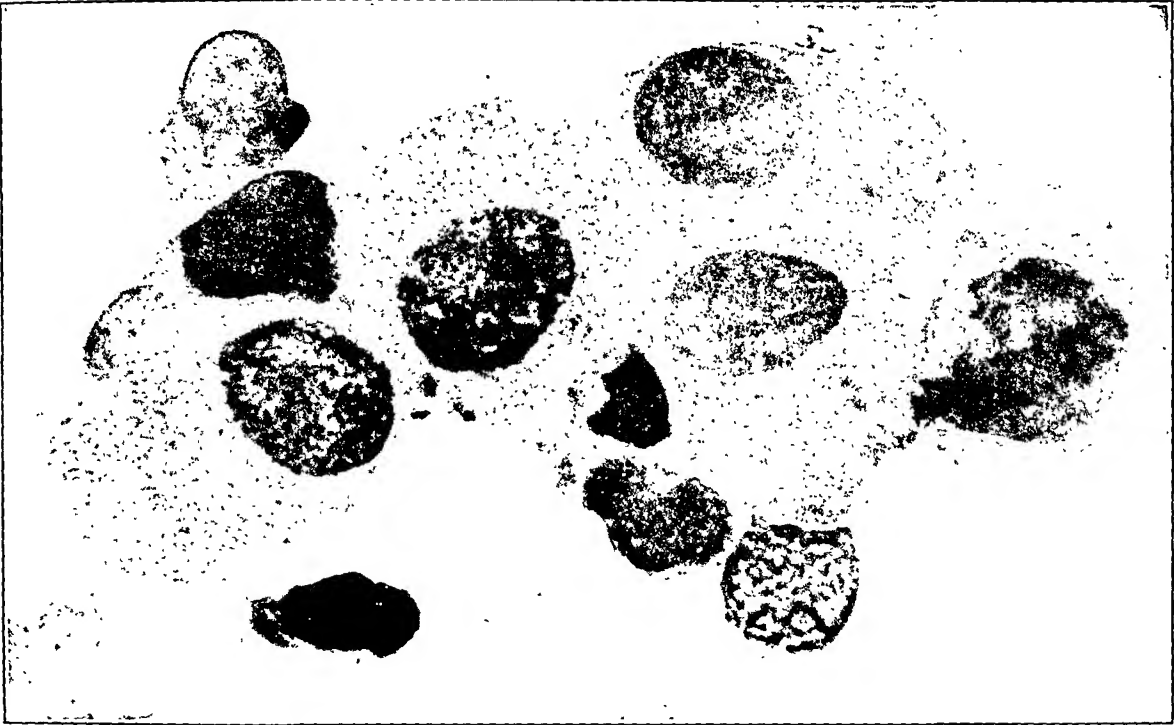


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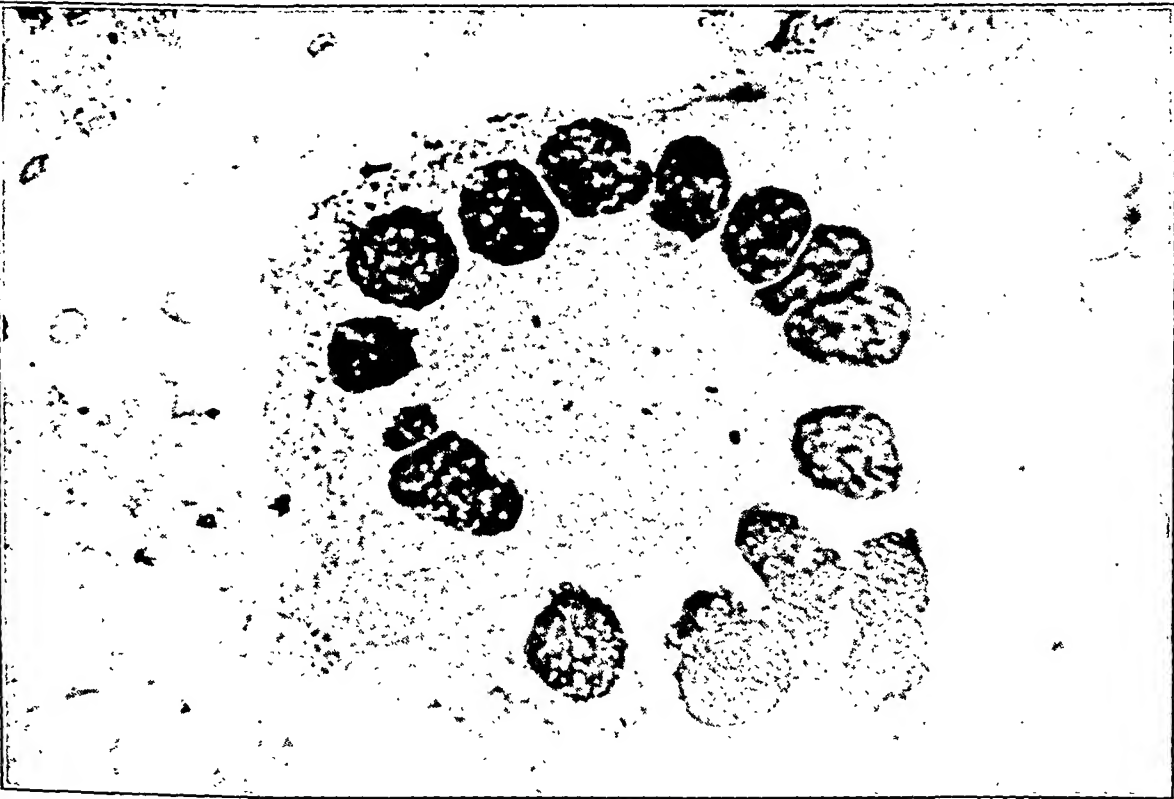
PLATE 90

FIG. 7. A clump of cells from the same preparation as in Fig. 6. The four large cells are typical "X" cells. Granules and fine vacuoles can be distinguished in their cytoplasm. The small cell in the lower left corner is a lymphocyte. The two medium sized cells in the upper left corner are "reticular" cells. The cell at the extreme right suggests an intermediate stage between a "reticular" and an "X" cell. Wright's stain. $\times 1000$.

FIG. 8. A multinucleated cell from the same preparation as the two preceding. As in Fig. 6, there is a darker area in the center of the cell suggesting the rosette area. This cell resembles a Langerhan's giant cell. Wright's stain. $\times 1000$.



7



8

FIBROSARCOMA OF THE PLEURA *

REPORT OF A CASE

H. E. MACMAHON, M.D., AND G. K. MALLORY, M.D.

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A malignant tumor of fibroblastic origin arising in the pleura is very unusual. In reviewing the literature to 1908, Robbins¹ briefly epitomized ten cases of pleural sarcoma. These showed a wide variation both in their gross morphology and in their pathologic histology, and occurred in patients whose ages ranged from three to sixty-seven years.

Robbins reported a case in which a sarcoma formed a thin but distinct layer over the entire surface of the left pleural cavity. This tumor invaded the lungs and metastases were found in the mediastinal lymph nodes and in the liver.

In another case, reported in 1916 by Pallasse and Roubier,² a large fibrosarcoma filled the right pleural cavity and invaded the diaphragm. These authors offer a classification of primary malignant tumors of the pleura, dividing them into sarcomas and endotheliomas, diffuse and circumscribed, and point out that a distinction may not readily be drawn.

During the past ten years little attention has been given to this condition by either clinicians or pathologists.

REPORT OF CASE

Clinical History: J. C. (Hospital number 544907), a white male, unmarried, fireman, 71 years of age, entered the Boston City Hospital on Aug. 13, 1927, complaining of a pain in the right side of his chest and shortness of breath. The family history is negative, and the past history, except for a loss of thirty pounds in weight during the preceding six months, is without interest.

His present illness apparently began about three weeks before entry when the patient was seized with an excruciating pain in the right side of his chest. This was relieved with rest and heat. After two or three days he noticed that on slight exertion he would become short of breath. Both of these symptoms persisted till the time of his admission into the hospital.

Physical Examination: An elderly man, well developed and well nourished. The findings, other than those relating to his chest, are irrelevant. The chest was asymmetrical; the movements, somewhat hurried and gasping, were rapid

* Received for publication May 1, 1928.

and shallow, with expiration moderately prolonged. The expansion on the right side was diminished. The left lung was resonant to percussion; the right, slightly resonant at the apex, was dull to flat over the remainder of the chest. Tactile and vocal fremitus on the right side were diminished over the greater portion of the lung and absent at the base. Breath sounds were exaggerated throughout the left lung, bronchovesicular over the right apex, and distant to absent from the level of the third rib down. There was a Grocco's triangle on the left side. The heart was enlarged or displaced. Apical impulse, distinctly palpable 15 cm. to the left of the midsternal line, was diffuse in character, but regular in force and rhythm. There were no murmurs. Blood pressure, systolic 100, and diastolic 65.

Treatment and Progress Notes: On day of admission, 1140 cc. of thin blood-stained fluid, with a specific gravity of 1.025, were aspirated from the right pleural cavity. The symptoms were appreciably relieved. Two days later, 300 cc. of dark, uniformly colored, bloody fluid, with specific gravity of 1.030, were removed from the same cavity. The patient's condition became worse and he died during the night.

Laboratory Findings: With the exception of the examination of the aspirated fluid the laboratory findings were negative. The first fluid showed 46 per cent lymphoid cells and 54 per cent polymorphonuclear leucocytes. Cultures and guinea pig inoculation were negative. Examination of the second specimen was negative for tumor cells.

Clinical Diagnosis: Cardiac decompensation; hydrothorax.

The autopsy was performed six hours postmortem. Tissue was immediately placed in Zenker's fixative and later embedded in paraffin and stained with eosin-methylene blue, phosphotungstic acid hematoxylin, and Mallory's aniline blue.

AUTOPSY REPORT

The right side of the chest is larger than the left. The right side of the scrotum is distended with a firm, solid, nodular, round mass, 8 cm. in diameter, which is neither adherent to the skin nor to the subcutaneous tissue.

Peritoneal Cavity: The diaphragm on the right is depressed to the level of the costal margin, whereas on the left it is at the fifth intercostal space. The lower margin of the liver is 11 cm. below the xiphoid process and 10 cm. below the costal margin in the right midclavicular line.

Pleurae and Lungs: The left lung and left pleura on gross examination are normal and may be passed without further comment. On the right, the parietal pleura is unevenly thickened and forms a large sac, measuring 30 by 20 by 20 cm., which is distended with 2200 cc. of thin blood-stained fluid. The lung is collapsed and forms

a small and very firm mass projecting from the medial wall of the pleural cavity.

The parietal and visceral pleurae, posteriorly and toward the mediastinum, are firmly adherent to one another. The visceral pleura, which is red and shaggy, is only slightly thickened except where small finger-like processes encroach upon the lung parenchyma. The inner surface of the parietal pleura is mottled yellowish brown and red, and presents a cobblestone appearance roughened by adhering flecks of fibrin. The fresh surface, which varies in thickness from 3 mm. to 4 cm., is firm, friable, glistening and gray. It strips easily from the ribs and intercostal spaces leaving a relatively smooth surface. Medially it is attached to the parietal pericardium, and inferiorly it is inseparably united to a thickened, hard and nodular diaphragm.

Pericardial Cavity: Surfaces are smooth and glistening. On the right side the pleura and pericardium are very adherent.

Heart: Weight 435 gm., is moderately enlarged and displaced to the left. The wall of the left ventricle is thickened; the coronary arteries are sclerosed.

Mediastinum: The mediastinal lymph nodes are normal in size, soft and deeply pigmented. A single node containing a minute, hard, white, glistening body, 2 mm. in diameter, lies adjacent to the thickened right pleura.

The remaining autopsy findings include moderate and generalized arteriosclerosis, a congenital cystic kidney, and an old infectious lesion of the right testicle.

The microscopic examination of the right pleura, right lung, peribronchial lymph nodes and diaphragm is of particular interest.

MICROSCOPIC EXAMINATION

Pleura: The entire right parietal pleura is infiltrated by tumor tissue composed of round and spindle-shaped cells and a variable amount of intercellular material. The morphology of the cells, their arrangement, their invasive character and their rapidity of growth suggest a fibrosarcoma. Toward the ribs and intercostal spaces, the tumor is very cellular and is invading the surrounding fat and fibrous tissue; here the cells are growing rapidly and forming very little intercellular substance. Nearer the pleural cavity where the tumor is growing more slowly the ratio of cells to collagen is re-

versed, there being few cells and much collagen. The inner surface of the cavity is composed of necrotic tissue covered by a layer of fibrin and poorly preserved inflammatory cells.

The structure of the visceral pleura varies in different places and generally is quite unlike the parietal layer. Over the greater part of the surface of the lung it is represented as a relatively thin and uneven layer of poorly preserved collagen with a few degenerating fibroblasts and endothelial leucocytes. In these areas the collagen has formed coarse strands which cross one another, run in different directions, and show no orderly arrangement.

In some areas where a continuous layer of elastic fibrils still indicates the position of the original pleura, collagen showing this same disorderly arrangement has been deposited on both sides of the pleura.

In a few places along the surface there is actively growing tumor tissue which has invaded the lung parenchyma. In such areas it is usually found that as the tumor is traced into the lung it gradually loses its cellularity and becomes more and more collagenous.

Lastly there are portions of the lung that are covered with a well preserved and orderly layer of hyaline connective tissue enclosing few foci of lymphoid cells and endothelial leucocytes.

Lung: The entire lung shows a varying degree of atelectasis, which is greatest at the periphery beneath the thickened pleura. The parenchyma of the posterior and inferior portion of the lower lobe is deeply invaded by actively growing tumor tissue. In this region the bronchioles are compressed and the alveoli are completely collapsed or have totally disappeared. There is a cellular infiltration composed largely of lymphoid cells and endothelial leucocytes throughout this portion of the lung, but this inflammatory reaction is most concentrated along the advancing margin of the tumor.

The alveolar spaces of the middle and upper lobes contain little precipitated albumen, few red blood cells, pigmented endothelial leucocytes and threads of fibrin. As a result of a hemorrhage, the alveoli of the lower lobe are filled with well preserved red blood cells.

Small patches of pneumonia in which the bronchioles and surrounding alveoli are filled with an acute inflammatory exudate are found in all three lobes.

The vessels everywhere are very congested and the walls of the larger arteries are thickened and sclerosed. The perivascular and

peribronchial lymphatics are distended with polymorphonuclear leucocytes, lymphoid cells and endothelial leucocytes. Many foci of lymphocytes lie in the scar tissue surrounding the larger bronchioles.

A rather unusual finding in the lung, and one which probably bears no relation to the sarcoma of the pleura, is the presence of a small group of alveoli, situated at some distance from the tumor, which are distended with blocks of cartilage of a primitive type suggesting a small chondroma.

Lymph Node: A small focus of tumor tissue is situated in the periphery of a single node and is extending by finger-like processes into the central portion. Here the tumor is growing rather slowly, there are very few mitoses and the cells are well differentiated and separated by a considerable amount of intercellular material. The lymph nodules show a rather aplastic condition while the sinuses are filled with endothelial leucocytes containing carbon pigment and hemosiderin.

Diaphragm: This is covered on the upper surface by a narrow layer of actively growing tumor tissue and in a few places is definitely invaded. The muscle fibers beneath and surrounded by the tumor are compressed; they are small, atrophic and degenerated and many have completely disappeared with consequent sclerosis of the surrounding stroma. Throughout the diaphragm there is a well marked chronic inflammatory reaction which is most marked near the tumor.

HISTOLOGY OF THE TUMOR

The type cell from which the tumor arises is the fibroblast. In this tumor it occurs most commonly as a long, well differentiated spindle-shaped cell. The nucleus is elongated, the chromatin forms a very delicate network, and the nucleoli appear as small round dots. Less frequently the cell is nearly round or oval; this type is probably less differentiated and is formed where the tumor is growing most rapidly; here, the nucleus is round or irregular, the network of chromatin is coarse, and the nucleoli vary in number, in size, and in contour. Between these two rather characteristic types there are many polymorphous forms. Single and multiple mitoses are fairly numerous.

Tumor giant cells, containing a single large nucleus or many nuclei, are sparsely distributed in the more cellular portions of the

tumor. Their nuclei show a wide variation in size and in shape. The chromatin is coarsely granular, and the nucleoli vary from small discrete dots to large irregular bodies. The cytoplasm of these cells stains more deeply than the smaller cells and extends out in streamers in many directions.

Fibroglia fibrils may be traced from the ends of the spindle-shaped cells, but they are not visible where the cells are more primitive and polymorphous.

Collagen fibrils, which form the intercellular matrix, vary in different parts. Where the tumor is growing actively and invading there is very little intercellular material, whereas in other areas where growth has practically ceased there is a great deal of collagen, and the fibrils have fused together to form wide irregular bands.

The grouping of the cells is inconstant. Sometimes the spindle-shaped cells lie in parallel rows, more commonly they are grouped in bands which course in any direction. The less mature cells show no definite arrangement except when clustered about the vessels.

The stroma in the cellular portion of the tumor consists of dilated blood vessels lined with a single layer of endothelium. In the more collagenous portions the vessels appear compressed and are surrounded by a definite ring of connective tissue.

Small areas are dispersed throughout the tumor in which the cells are undergoing degeneration or are already necrotic. These areas are always infiltrated with polymorphonuclear and endothelial leucocytes, and frequently, in the zones of necrosis, there is some hemorrhage and blood pigment suggesting a gradual infarction.

DISCUSSION

Clinically it seems remarkable that a tumor could extend throughout the entire pleura, produce such an effusion, and lead to such a degree of atelectasis without producing more discomfort, since from the clinical history it is apparent that we are describing a patient who had no symptoms until three weeks before entry into the hospital. Furthermore, the clinical impression of the case as being one of cardiac decompensation and hydrothorax suggests the difficulty of diagnosis.

From the view point of the pathologist the tumor is unusually interesting, first because a fibrosarcoma of the pleura is very rare,

and secondly, because one forming a complete sac of the pleural cavity is indeed a curiosity.

It would be difficult to suggest how or from what part of the pleura this tumor had originated. There is some evidence that there has been an old healed pleuritis since many portions of the thickened visceral pleura may be readily considered as simply the result of an old inflammatory process.

The most actively growing portion of the tumor is along the outer surface of the parietal pleura, whereas along the internal surface of the parietal pleura and in the visceral pleura growth has almost ceased. It was pointed out above, that small areas are scattered throughout the more cellular portion of the tumor which show degenerative changes frequently associated with some hemorrhage and blood pigment. Probably these are simply early retrograde changes in the cells secondary to a diminished circulation, and such an explanation is consistent with the varied histologic pictures which different portions of the tumor reveal.

In general, the tumor may be classed as a slowly growing fibrosarcoma showing little tendency either to invade or to produce metastases.

SUMMARY

1. A case is reported of a primary fibrosarcoma of the pleura in an elderly male.
2. The literature is briefly reviewed.
3. The case is discussed as an unusual pathologic condition which warrants clinical consideration.

We are indebted to Dr. F. B. Mallory and Miss Catherine G. Norton for the illustrations and to Miss Marion E. Lamb for technical assistance.

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DESCRIPTION OF PLATES

PLATE 91

FIG. 1. The right pleura has been incised and the halves spread out to show the cobblestone appearance of the inside of the cavity, and the small atelectatic lung.

FIG. 2. Portion of the tumor showing the spindle-shape morphology of the cells, and their parallel arrangement. One mitotic figure. $\times 1000$.



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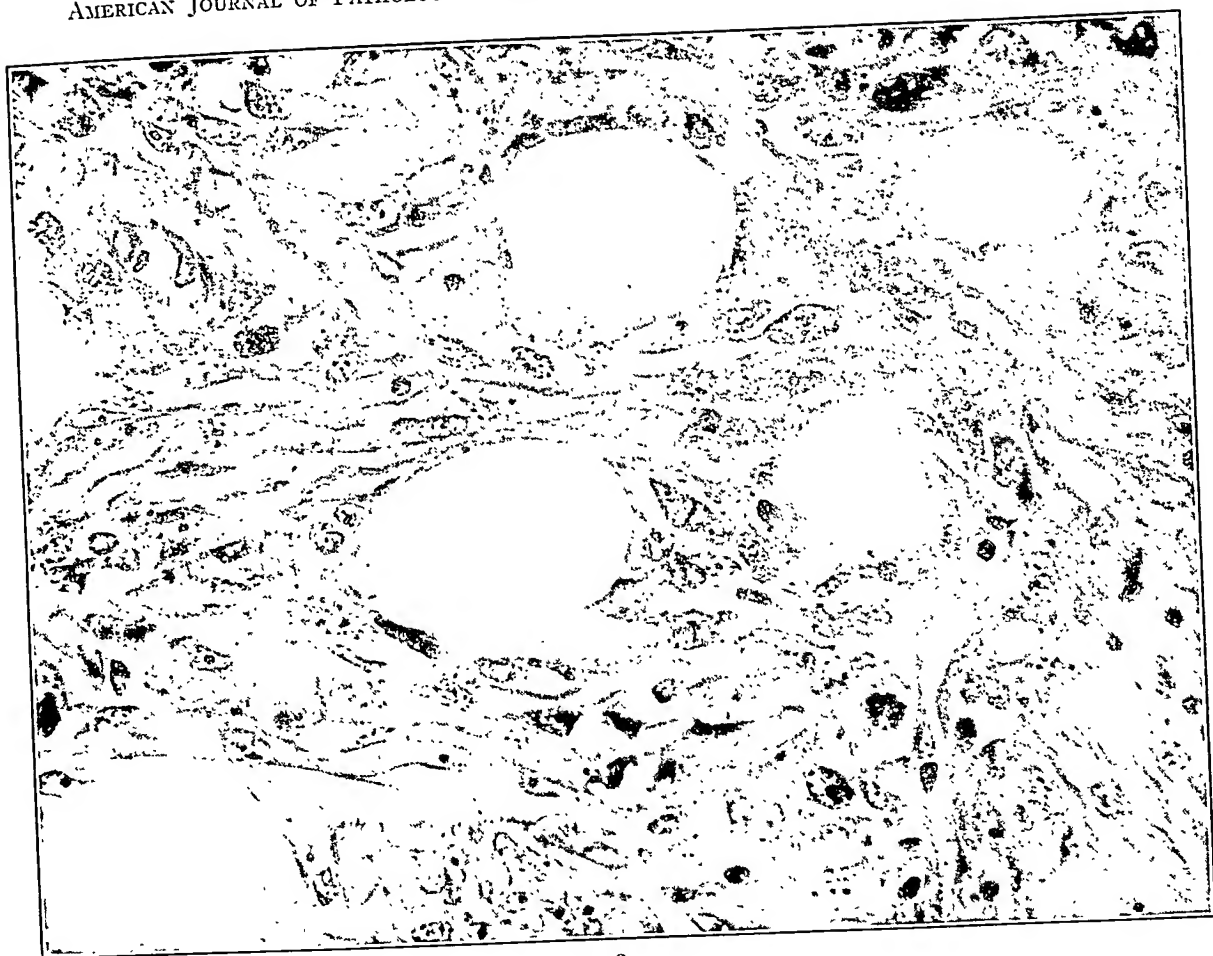
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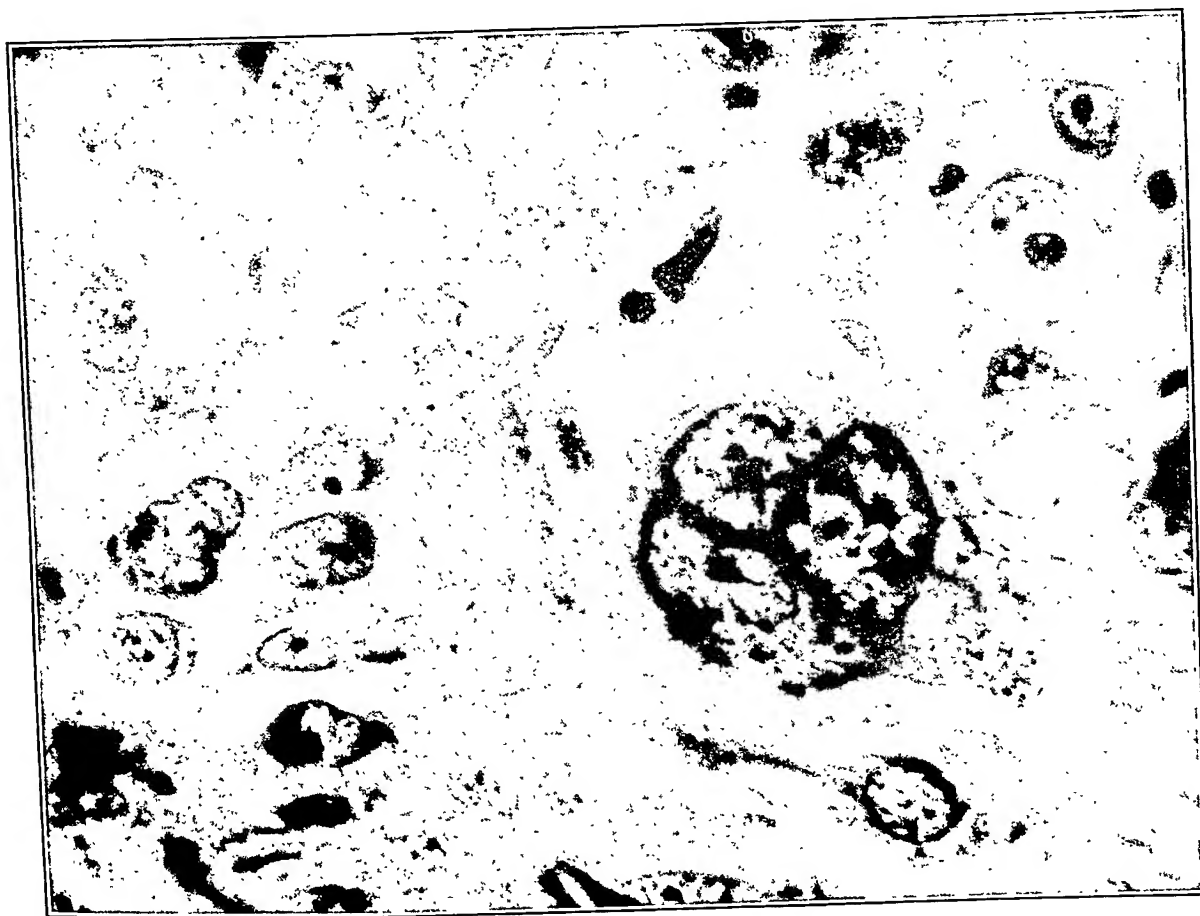
PLATE 92

FIG. 3. Invasion of fat by tumor. An actively growing portion of the tumor showing several mitoses. $\times 500$.

FIG. 4. An area in which the cells are oval and round. Single and multinucleated giant cells. $\times 1000$.



3



4

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THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHESUS* *

I. GROSS PATHOLOGY

N. PAUL HUDSON, M.D.

INTRODUCTION

This report is based on the necropsy findings in sixty-eight *Macacus rhesus* monkeys fatally inoculated with the strain of yellow fever virus obtained from Asibi, an African native. A brief clinical history of this patient has been presented in an earlier report,¹ where also the gross pathology of a few individual animals was described and a brief summary of the general findings given. This and other articles to follow on the pathology in the *M. rhesus* are intended to amplify the earlier report.

At the time of writing, Jan. 1, 1928, eighty-eight animals of this species were inoculated in various experiments connected with the study of the virus. Eleven did not succumb; six of these were protected with human convalescent yellow fever serum, one was injected with washed red blood cells, one was bitten by apparently non-infective mosquitoes, and three were evidently insusceptible. Of the seventy-seven monkeys that died, nine fatalities were obviously not due to experimental infection, but are to be accounted for as follows: two were killed on the first day of fever, two died of pneumonia, two of generalized tuberculosis, two of dysentery and one of acute sepsis. This analysis reveals that sixty-eight animals were experimentally infected and available for this study.

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

Received for publication May 19, 1928.

The transmission of the virus was accomplished in various ways. One rhesus monkey died after inoculation with the patient's blood; twenty-five after inoculation with infectious monkey blood; two with serum and five with serum filtrate, monkey to monkey transfer; twenty-six after being bitten with infective *Aedes* (*Stegomyia*) *aegypti*; and nine following miscellaneous experiments, such as injection of mosquito emulsions and of blood stored for various periods of time.

For the purpose of a control, two normal rhesus monkeys in excellent condition were killed and studied. In addition, numerous other uninoculated monkeys of this species dying of natural causes were examined. These causes of death may be classified as dysentery, tuberculosis, pneumonia and undetermined; at no time, however, did the prevalence or incidence of any one disease suggest the existence of an epidemic. No gross lesion was found in these animals similar to yellow fever pathology, except in one or two animals dying from dysentery, in which a few petechial hemorrhages were seen in the gastric mucosa.

The experimental animals were apparently full-grown adults, although their ages were undeterminable. In this series, males were more numerous than females. Their lengths over head and trunk varied from 35 to 50 cm., averaging about 40 cm., and their weights were from 1600 to 3000 gm. Animals obviously sick were avoided in experimentation as much as it was possible, and as a rule monkeys coming to postmortem examination were fairly well to very well nourished.

Diseases of recognizable pathology concurrent with the experimental infection were found in a relatively few animals. One monkey (*M. rhesus* No. 253 A) had tuberculosis of the lungs, while one (No. 334) had generalized tuberculosis; a mild bronchopneumonia was present in one (No. 384); another animal (No. 349) showed a localized peritonitis; and a sixth rhesus (No. 229) had evidence of an acute pancreatitis. Microscopic examination of these animals has confirmed the existence of the complicating gross lesions, together with a yellow fever infection, except in the case of No. 349 which has not been studied microscopically. In the case of No. 229, it cannot be proved whether yellow fever or the acute pancreatitis caused death, but ample gross and microscopic evidence is manifest to indicate the presence of a severe yellow fever infection.

POSTMORTEM OBSERVATIONS

Jaundice: Jaundice appeared as a light yellow to lemon yellow color with sometimes a tendency to a greenish yellow shade, especially in the case of body fluids. The color was not brownish or like that seen in obstructive jaundice in man.

A yellowish tint of the skin was best seen in the upper part of the face and eyelids but, in this group of animals, discoloration of these parts was irregular and mild. It was most evident in those animals that were examined some time postmortem.

Jaundice of the tarsal conjunctivae was present distinctly or markedly in thirty and was mild in an additional seventeen of the sixty-eight monkeys. Just antemortem, capillary congestion of the conjunctivae often prevented the determination of jaundice which became evident after death with the emptying of the vessels. The subcutaneous tissue and fat were definitely icteric in thirty animals, while the larynx was regularly colored light yellow to deep lemon yellow. Jaundice was noted as distinct in the trachea of twelve and as mild in an additional eighteen. Icteric omental fat and gastric mucosa were seen five times each and this characteristic was noted in the external surface of the stomach fourteen times. Commonly the cleaned mucosal surface of the intestines was pale and yellowish in the absence of congestion or hemorrhages.

Often the peritoneal fluid was increased sufficiently to note its color, which was always yellow or greenish yellow. The pericardial fluid had, as a rule, the same abnormal coloration. The urine, when present, was regularly greenish yellow, which often was markedly intense.

Two parts almost invariably jaundiced were the larynx and the surface of the large vessels extending from the heart. In the case of the aorta, fifty-six were noted as being definitely to markedly jaundiced, seven were mildly jaundiced, while only two were negative and three are unrecorded. Here the color varied from a mild yellow tint to a deep lemon yellow and was most intense at the base of the aorta.

Jaundice of most parts was recognizable, although confusion was possible with the normal color of fat. The tarsal conjunctivae, aorta, trachea and larynx were dull or pearly white in the normal rhesus and in animals dying of natural causes. Peritoneal and pericardial

fluids and urine were colorless in control animals, except occasionally when the urine of sick, non-experimental monkeys was yellowish.

The marked irregularity in occurrence and degree of icterus is to be noted. The more intense jaundice was usually found in animals that either ran a long clinical course or were examined several hours postmortem; this relation, however, was not constant. There was no regularity in the parts involved except in the case of the larynx, aorta and other large vessels near the heart.

Pallor: This characteristic was commonly seen in the buccal, gastric and intestinal mucosa. Certain organs were likewise pale, notably the liver, kidneys and, to a certain degree, the heart.

Hemorrhage: The tendency to hemorrhage was manifest in various ways. Blood was definitely found at the base of several teeth, usually the upper incisors, in eleven animals, and in two additional monkeys the amount was so small as to make the finding uncertain. These eleven cases of positive bleeding gums were found in the absence of pyorrhea or evidence of trauma, and are exclusive of those caused to bleed antemortem by gently rubbing the lip over the gum. The gums just antemortem were often soft and purplish, and bleeding was induced in seven of twelve animals so examined. Twenty-two uninoculated monkeys were similarly treated and after prolonged and vigorous rubbing of the lips over the gums, blood appeared oozing from the margin of the gums in two animals showing gross evidence of pyorrhea.

Petechial hemorrhages occurred on the pleural surface of the lungs in about two-thirds of these experimental monkeys (Fig. 1). Somewhat fewer specimens displayed hemorrhages in the lung tissue, although it was not practical to expose much tissue by sectioning. The hemorrhages appeared as bright red dots 2 to 4 mm. in diameter and in number were from a half to two dozen on the surface of both lungs, usually closer to the upper figure.

It was very unusual to find any tendency to hemorrhage in the heart, and this was never observed in the musculature or epicardium. Twice, however, irregular diffuse hemorrhages were present in the endocardium.

The stomach in twenty-six, or a little over a third of these monkeys, contained variable quantities of altered blood (Fig. 2). The blood appeared as fine black or chocolate-colored threads and flecks, and when present in large amounts, blackened the whole stomach

contents. Usually the streaks of blood could best be seen in the mucus next the mucosa. Sometimes fresh red blood was found streaking from the mucosa, but in no instance could any gross lesion be demonstrated in the mucosal surface. Hemorrhage and congestion of the gastric mucous membrane were present seventeen times and usually not in the same animal. These characteristics were variable in degree, and the scattered and minute petechiae were largely in the fundus while the congestion occurred principally near the cardia. Altered blood in the stomach contents was generally independent of hemorrhage and congestion, and conversely, the latter two findings were not commonly concurrent with the black vomitus.

The lack of association of blood in the contents with hemorrhage or congestion of the mucosa, was again demonstrated in the small intestine. In twelve instances, mucosal changes were evident, while altered or fresh blood was found in thirteen specimens. These findings were present more often in the ileum than in the duodenum or jejunum, although occasionally unequally distributed throughout the small intestine. The quantity of altered blood varied from a definite smearing of the mucosal surface with partially altered blood to large masses of brownish red or chocolate-colored contents. No break in the continuity of the mucosa and no evidence of intestinal infection were found in these cases. Although sometimes present in the same animal, there was no definite relation in the occurrence of the hemorrhages or blood content of the stomach to those of the small intestine.

No hemorrhages were seen in the skin, peritoneum, retroperitoneal or perinephritic tissues, kidneys, liver, bladder mucosa, lymph nodes, brain or cerebral meninges.*

In the control animals, the tendency to hemorrhage or accumulation of altered blood in the gastro-intestinal tract was not observed except in two monkeys having manifestations of a dysenteric infection, in which petechial hemorrhages were present in the gastric mucosa.

Lungs: The lungs were always soft, pink and air-filled and the petechial hemorrhages were conspicuous against the pale background. Practically every specimen showed parasitic cysts of *Pneu-*

* Although in this series no hemorrhages were found in the adrenal glands in gross, they occurred in this organ in two monkeys inoculated with another strain of this virus.

monyssus griffithi.* These cysts were 3 to 5 mm. in diameter and had a soft yellowish center; they lay just under the visceral pleura and less commonly within the lung tissue. Microscopically, these areas contain a cyst with a definite wall and are probably connected with a bronchiole since some such cysts are lined by a layer of epithelial cells. The wall is densely infiltrated with endothelial leucocytes, lymphocytes, polymorphonuclears and a few eosinophiles. Cross-sections of a mite are sometimes observed in the cysts. In gross, narrow bands of hemorrhage often surrounded the cysts in the experimental monkeys, while in control animals these areas were almost colorless except for the yellowish center.

Heart: The weight of this organ varied with the size of the animal. Jaundice of the lining of the large vessels leading from the heart has been discussed. In addition, numerous specimens had a yellowish tint of the endocardium, auricles and valvular rings. Pallor of the musculature in some instances was conspicuous.

Liver: The weight of the liver varied somewhat but was roughly in proportion to the size of the monkey. There was no relation between the weight and degree of infection.

The color of this organ was the most conspicuous feature. When the abdomen was opened immediately after death, the liver was pale, pinkish yellow which faded with exsanguination to a markedly pale, yellowish brown or cream color (Fig. 3). If the animal had been dead a short time when examined, the liver was commonly very pale and cream-colored, with the surface sometimes splotched with red that blanched on cutting the large vessels and on handling. On section, the liver tissue usually had the same color as externally. This constant color characteristic gave the liver the so-called "box-wood" appearance, and indeed, the color generally matched a box-wood ruler at hand. The pallor and color of the liver were in sharp contrast to the deep red-brown liver of control animals.

Mottling of the surface with fine lobular markings was observed in most specimens, although often the liver surface was of a uniform pale cream color, especially on section. These lobular markings were best seen with a hand lens on the external surface; as a rule, a fine

* Mrs. Sophia Connal of the British Medical Research Institute, Yaba, very kindly identified these parasites as being *Pneumonyssus griffithi*, Order Acarina: mites. They correspond in every way to the parasite described by Gay and Branch² occurring in the *Macacus rhesus* and called by them *Pneumonyssus foxi*.

red dot representing the central vein, surrounded by a wide yellowish zone and a red interlacing periphery could be made out. Hemorrhagic areas were not demonstrable externally or within the liver tissue.

The surface of the liver was smooth and regular and the edges sharp. By external examination, it was about as firm as normally, but on section, the tissue was often soft and friable, and sometimes cheesy in consistence. The surface of the sectioned tissue was smooth and glassy, reflected light and generally appeared fatty. There was an obvious diminution in the amount of blood in the liver, and on sectioning, the tissue was dry and almost bloodless.

The gall-bladder was not remarkable. All specimens contained 3 or 4 cc. of more or less thick, mucoid, dark green bile. No resistance was offered to the flow of bile on pressing the gall-bladder and the intestinal contents were always bile-stained.

Spleen: The weight of the spleen varied considerably, but on the whole there was an increase over the weight of this organ in the control animals. The spleen was firm, its edges generally rounded and its surface smooth and normally colored. On section, this organ was dark red and firm, and blood did not ooze freely from the surface. Normally, the malpighian corpuscles are large, gray, prominent and closely dotting the splenic tissue. In the experimental monkeys, these bodies were regularly reduced in size, sometimes being barely visible, and separated by red pulp.

Kidneys: These organs were increased in weight over those of the control monkeys. Externally they were firmer than normal and, while encapsulated, somewhat pale and bluish red; often there was also a definite brownish tinge externally. The capsule stripped readily from a smooth pale brown surface. On section, the cortex and pyramids were always pale. The cortex was either light reddish brown or yellow-brown and the medulla was usually of the same color, although occasionally the pyramids were redder than the cortex. Sometimes the cortical capillaries were traced, but as in the liver, very little blood oozed from the tissue. A constant characteristic was the presence of fine, dull gray cortical rays easily seen with the naked eye and contrasted to the surrounding pale brown tissue. The cortex was firm and appeared full and tense. The glomeruli could not be made out. The pelvis and ureters were not remarkable.

Adrenal Glands: These structures were not affected in gross in this group of experimental animals aside from occasional congestion.

Pancreas: The pancreas was normal, with the exception of the one case (No. 229) mentioned above, in which there was an acute pancreatitis of unknown etiology.

Gastro-Intestinal Tract: The esophagus was not remarkable except for an occasional smearing with vomitus and frequent mild jaundice of a pale mucosa. The pathology in the stomach and small intestine has been discussed in connection with the subjects of hemorrhage, jaundice and pallor. The large intestine deserves mention because of the frequent occurrence of streaks of bright red blood on the surfaces of the mucosa and feces. No significance as regards experimentation is attached to this finding because of the occasional presence of round worms both free in the intestinal lumen and embedded in the mucosa. Worms were also extracted from submucous and, very rarely, subserous cysts filled with purplish blood. These worms on examination agreed with the description recently given by Branch and Gay³ of worms found in the *M. rhesus* and which they identified as being *Oesophagostomum brumpti*. The parasites were not constantly associated with diarrhea and their pathogenicity is yet to be determined.

Lymph Nodes: Axillary and inguinal nodes were occasionally congested and slightly enlarged, but as a rule these structures were small and pale. Mesenteric lymph nodes varied in size and were pale, yellowish pink. Peribronchial and peritracheal nodes were normal except in the rare instances of pulmonary tuberculosis. No hemorrhagic nodes have been seen.

Urinary Bladder: The bladder mucosa often appeared yellowish, and hemorrhage or congestion was never observed. The urine was highly colored and either yellow or greenish yellow. It regularly gave positive tests for albumin and bile and often showed hyaline and granular casts.

Genitalia: Jaundice of the vaginal mucosa was the only significant change found in the female genital tract. Similarly, the prepuce of males was generally icteric; otherwise, the male genitals were normal.

Brain: The brain was removed and weighed in several animals. It appeared normal externally and on section, and the weight varied directly with the size of the monkey. The cerebrospinal fluid was tinged with yellow in a few instances.

Other Parts: The organs of the neck, submaxillary glands, voluntary muscle and costal cartilages were not remarkable. The tongue was pale but otherwise normal. In instances of extreme jaundice, the tendons of large muscles and bursal surfaces were distinctly yellowish.

WEIGHTS OF ORGANS

The liver, heart, kidney and spleen were routinely weighed. Weights of organs from sixty-three monkeys are available for analysis and the length over head and trunk is taken for the basis of comparison. When all experimental monkeys are compared with the

TABLE I
Weights of Organs

	Number of animals	Length over head and trunk	Liver	Heart	Kidney	Spleen
		cm.	gm.	gm.	gm.	gm.
Average in normal monkeys (killed) . .	2	43	84	16.5	13	4.5
Average in monkeys dying of natural causes	17	40.3	77.7	10	14	4
Average in all non-experimental mon- keys	19	40.6	78.4	10.6	13.8	4
Average in experimental monkeys . .	63	40.6	83.2	11.7	17.5	5.5

normal animals, the average weight of the kidneys is 42 per cent and that of the spleen is 22 per cent over the corresponding weights in the normal, even though the average length of the experimental animals was less than that of the normals. The comparison of weights of organs with only two animals is obviously open to error. Another available method of determining any variation in organ weights of experimental animals, is to compare their weights with those of seventeen rhesus monkeys dying from miscellaneous, non-experimental causes. When this is done, we can compare animals of the same average length and find that the kidneys are 27 per cent and the spleen 37 per cent heavier in experimental monkeys than in the other group. The heart and liver are practically the same in the two groups, although slightly heavier in the experimental series.

Obviously, under the circumstances, no mathematical statement of the increase in weight of the kidneys and spleen of experimental monkeys can be made as a group, but many individual animals illustrated the point that in the animals dying of yellow fever, the weight of these organs is generally increased. A few examples are included in the accompanying tables which summarize the analysis of organ weights.

TABLE II
Weights of Organs of Selected Individual Monkeys

Animal No.	Length over head and trunk	Liver	Heart	Kidney	Spleen
	<i>cm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
312.....	42	74	13	20	7
316.....	39	92	8	18	7
325.....	42	89	16	18	8
339.....	48	140	19	31	9
342.....	36	65	10	17	7
360.....	42	100	14	20	7
363.....	43	73	13	19	7
391.....	37	64	10	15	6
Average	41	87	13	19	7

SUMMARY

The gross pathology in sixty-eight *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of yellow fever virus is described. The variety of routes and modes of infection furnished ample opportunity to study the effect of the method of transmission of virus on the pathology produced. As yet, however, no variation in the gross findings has been observed attributable to the method of infecting the animal, and all variations noted are within the limits found to obtain in a large number of animals infected by any one method, *e. g.*, intraperitoneal injection of blood.

The principal gross lesions were jaundice, hemorrhage and pallor of various parts, and the changes in the liver, kidney and spleen. Jaundice and hemorrhage were variable in occurrence and degree, but the pallor of the liver and kidney and the buff or yellowish "boxwood" color of the liver were constant and definite. The surface of sectioned liver tissue appeared glassy and fatty and the tissue

was generally friable, almost bloodless, and obviously necrotic. The spleen and kidneys were commonly increased in weight over those of control animals. This seemed to be due in the case of the spleen to congestion and in the kidneys to an apparent acute degenerative change.

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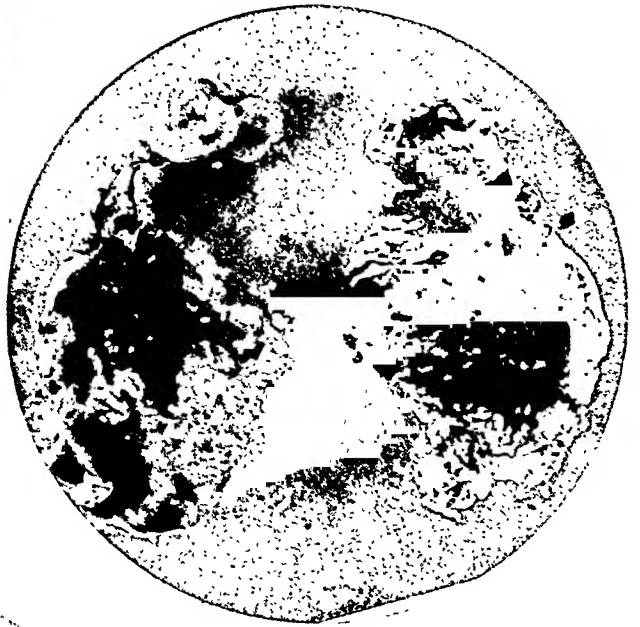
DESCRIPTION OF PLATE

PLATE 93

- FIG. 1. *M. rhesus* No. 338. Petechial hemorrhages of the lungs. This specimen shows an unusually large number of hemorrhages. It also demonstrates about a half dozen cysts of *Pneumonyssus griffithi* each surrounded by a zone of hemorrhage; the cyst at the apex of the right lung is best illustrated. Three-fourths natural size.
- FIG. 2. *M. rhesus* Nos. 316 and 368. Two opened and partially everted stomachs, showing altered blood ("black vomitus") covering the mucosal surface. Three-fourths natural size.
- FIG. 3. *M. rhesus* No. 411. Contrast of pallor of liver of experimental monkey (left) with deeply colored liver of uninoculated monkey (right). Note that the difference in size in organs was due to the difference in size of the animals, the experimental monkey being 38 cm. over head and trunk, while the other, dying of dysentery, was 44 cm. Both specimens removed at the same time, were washed in water, immersed in Kaiserling fluid and photographed at once. About one-third natural size.



1



2



3

Hudson .

Pathology of Experimental Yellow Fever in *Macacus Rhesus*, I

THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHEBUS* *

II. MICROSCOPIC PATHOLOGY

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INTRODUCTION

This report on the histopathology of experimental yellow fever is based on the microscopic study of tissues of thirty *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of the virus.¹ All these animals are included in the report on the gross pathology² found in the sixty-eight monkeys of the same species.

The virus was transmitted in various ways to the animals microscopically studied. These methods may be grouped in the following manner:

Blood from patient (Asibi)	1
Blood and organ emulsion from monkey	1
Blood from monkey	8.
Blood from monkey applied to scarified skin	1
Monkey serum filtrate (Berkefeld N)	1
Monkey serum filtrate (Seitz asbestos)	1
Mosquito (<i>Aedes aegypti</i>) transmission	16
Emulsion of mosquitoes (<i>A. aegypti</i>)	1

Monkeys were examined and tissues preserved as soon after death as possible, because of rapid postmortem changes. The necropsy was done at once in the case of twenty animals, within one hour after the death of seven, and a few hours postmortem in three. In a few instances, chloroform was used to hasten the end of moribund animals in order to make the postmortem examination by daylight and, compared to the large number of animals not so treated, the small amount of chloroform needed did not seem to alter the microscopic pathology.

The diet of all monkeys has been rice, oranges, bananas, bread, evaporated milk and water. For purposes of comparison, the tissues of two apparently normal and several rhesus monkeys dying from natural causes (dysentery, tuberculosis and undetermined) were available.

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

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The tissue fixatives employed were 10 per cent formalin and Zenker's fluid and the staining method was routinely hematoxylin and eosin; occasionally van Gieson and Giemsa preparations were studied. For the demonstration of fat, frozen sections were stained with scarlet red and counterstained with hematoxylin.

PATHOLOGIC HISTOLOGY

Liver: This organ furnishes the most extensive and constant pathology and shows chiefly fatty degeneration and necrosis.

No fat is found in one normal rhesus and in the other, it occurs as large droplets sparsely sprinkled through the lobule. In monkeys dying from natural causes, there is only a small amount of periportal fat. In paraffin sections of the experimental tissues, large and small vacuoles are evident, especially in the normal or moderately altered liver cells, such as in the periportal region. In frozen sections, fat is demonstrated in every liver. In the majority of specimens, it is present in extreme amounts, occurring as minute to large droplets in practically every parenchymatous cell. In numerous instances, more fat, usually as larger droplets, is found in the periportal and central zones of the lobule. In one liver (No. 304) in which the necrosis is mild, fatty degeneration is limited to the midzone.

In the normal liver, the parenchymatous cells are regular, polyhedral and arranged roughly in columns; the neutrophilic cytoplasm is not uniformly stained and gives a positive test for glycogen (Best's carmine stain). The cytoplasm of the liver of monkeys dying from natural causes is smoothly stained and neutrophilic. In contrast to these control animals, the hepatic cells of experimental monkeys are not arranged in columns in the affected regions, but are jumbled, rounded and irregular in size and shape. No glycogen is found in these sections (one monkey tested).

Early degenerative changes are represented by the loss of the normal cell relation and the presence of mild to marked eosinophilic-staining properties. More advanced stages of necrosis appear as acidophilic, coarsely granular cytoplasm in distorted cells. Finally, disintegration takes place and the cytoplasm is either intensely or poorly stained, and granular; the cell wall may be entirely indefinable. In about two-thirds of the livers, hyaline degeneration is one of the forms of cytoplasmic change. Hyalin is seen as small, irregular

homogeneous, well-stained masses within the cell and occurs in only a relatively few cells of the section.

Nuclear changes coincide with the changes of the cytoplasm. Large, poorly stained, vesicular nuclei with prominent nucleoli are found in the necrobiotic liver cells. In such nuclei, minute, dull red granules are often found. They were present in half the thirty specimens studied; in an additional six specimens, this type of nuclear change is less often present. Occurring in half the livers of this group, variable numbers of nuclei appear as small, round, intensely acidophilic dots. This form of degeneration is usually seen in the comparatively rare, small liver cell having smooth, intensely stained cytoplasm. The nucleus finally disappears in advanced necrotic cells either by lysis or karyorrhexis. In eleven specimens, karyorrhexis is common or marked, while in five others it occurs relatively infrequently. When karyorrhexis is marked, scattered nuclear debris is conspicuous in the hepatic cells throughout the section. Mitosis is not found.

The degree of necrosis varies only slightly among the specimens and in only one instance (No. 304) is it limited to the early stages of degeneration. No effort has been made to determine the variation in degree of necrosis throughout the liver, but there is reason to believe that such exists. The variation in any one section, however, is negligible, all lobules being equally affected.

The extent of necrosis and necrobiosis in the lobule, on the other hand, is not constant among the specimens except that the midzone is always attacked. In two cases, the midzone alone is necrotic, mild in one (No. 304) and extreme in the other (No. 312). In one-third of the livers a wide intermediate zone encroaching upon the periportal and central zones is involved; in an additional third, parenchymatous cells of all zones are degenerated, leaving only a fringe of intact but vacuolated cells about the portal region and central vein; and in the other instances, a border of periportal cells alone is undegenerated. In the majority of cases of extensive necrosis, the most extreme changes are midzonal. Under such circumstances, the most numerous necrotic cells are in the intermediate zone, while toward the limiting structures the necrotic cells, mingled with the necrobiotic cells, decrease and the latter type increase in numbers. The same is true of necrobiotic cells in relation to the intact and normal cells. In no case is either central or periportal necrosis alone encountered.

In spite of disintegration of parenchymatous cells, no collapse of cell space is evident and lobules retain their normal shape and size.

Sinusoids are easily followed except when distorted in extremely necrotic areas. Their lining cells are rarely enlarged and only occasionally phagocytic. The nuclei of these cells are well stained and sometimes comprise the only intact nuclei of a necrotic region. Congestion is not a conspicuous feature and when present is irregular and mild. No thromboses are evident. Hemorrhage is rare and is found to an appreciable degree in one instance in the midzone and in another about the central vein. On the other hand, a few to numerous scattered red blood cells are seen to be extravascular in one-third of the specimens.

Portal areas are commonly not remarkable aside from mild and variable lymphocytic infiltration. Often endothelial leucocytes and occasionally polymorphonuclears are found in these regions and in one instance necrosis is obvious. There is no fibrosis.

In this organ, inflammatory cells are generally present in the necrotic regions but their presence and numbers do not depend on the degree or extent of parenchymatous necrosis. In twenty-four specimens, scattered polymorphonuclear leucocytes are in the sinusoids, and in three of these, they occur in foci as well; in half of these twenty-four instances, these cells are also among or in necrotic liver cells, but not in large numbers. In about the same number of animals, not always, however, in the same specimens, endothelial leucocytes are found, both intravascular and extravascular. In the latter situation, they are occasionally large and phagocytic, sometimes containing acidophilic granular debris. While inflammatory cells commonly accompany the necrosis of parenchymatous cells, their occasional complete absence indicates that they do not constitute an essential part of the pathology. Lymphocytes and eosinophiles are not found.

Kidney: Fatty degeneration is not demonstrable in this organ of the normal and uninoculated monkeys. In paraffin sections of numerous experimental tissues, fine vacuoles are seen near the base of epithelial cells of cortical tubules. In frozen sections stained for fat, large amounts of this substance are demonstrated as minute to small droplets in the tubular epithelium of twenty-seven of the thirty specimens. The convoluted tubules show the most advanced fatty degeneration and when less than extreme amounts of fat are present,

the straight tubules are spared. Fat is not stained in the glomerular structures or in parts other than the tubular epithelium.

In non-experimental animals, the renal tubular margin is slightly irregular, but the lumen is distinctly patent. In experimental sections, the epithelial cells of convoluted tubules, more than of straight tubules, are swollen, slightly granular and occasionally acidophilic. In twenty-three specimens, this acute degenerative change is marked almost to the occlusion of the lumen of the convoluted tubules; it is mild in three others and inconspicuous in three. Necrosis is not extensive in these monkeys; however, a relatively few to numerous necrotic epithelial cells of cortical convoluted tubules are evident in the majority of cases. Such cells are granular, disintegrated and without nuclei, and in the presence of one or two necrotic cells in the cross-section of a tubule, the margin is ragged.

The nuclei of these cells showing cloudy swelling are usually well preserved, although in a few instances some are poorly stained. Ten specimens demonstrate pyknotic nuclei of epithelial cells, which are more generally found in straight than in convoluted tubules. Karyorrhexis is relatively uncommon and is conspicuous in only two instances.

No inflammatory cells are found in kidney sections, either in degenerating cells or in the tubules. Congestion is not marked and when present, occurs mildly and irregularly. There is no indication of hemorrhage and no red blood cells lie in the tubules.

Tubules of the cortex and medulla as a rule contain varying amounts of granular or circular débris. Few to many granular and hyaline casts are in straight and collecting tubules of about one-half the specimens. The two varieties are not always in the same animal, although as a rule they are coincident. Sometimes they are distinctly bile-stained.

In six animals, deposits of hematoxylin-stained material are seen in collecting tubules and less frequently in convoluted tubules. They are best demonstrated in formalin-fixed tissues as either clumps of minute granules, larger solid masses, or discs. Their color varies from slate-blue to almost black. In frozen sections counterstained with hematoxylin, they are conspicuously darkly stained. After treating paraffin sections with silver nitrate according to Klotz' method, these deposits appear dark brown or black. Immersion in a weak solution of nitric acid before staining makes it difficult to

demonstrate the deposits with hematoxylin; those found are only faintly colored, whereas cell nuclei are not altered in their staining properties. With these findings and the demonstration of form, location and hematoxylin-staining, it seems that such casts are calcareous deposits. At least one source of this variety of cast may be a form of degeneration of the epithelium of collecting tubules, since one can trace similar intracellular masses through the changes of the cell to its necrosis and disintegration. Sometimes these calcareous deposits form a rough ring about the lumen of the collecting tubules, either within or on the surface of necrotic cells or in the entire absence of epithelium.

Glomerular capillaries are sometimes congested and their spaces occasionally contain a small amount of granular débris; otherwise these structures are not remarkable. The renal capsule and pelvic mucosa are unaltered.

Heart: In comparison to the normal controls, paraffin sections show little change in cellular structure. In a few specimens, the fibers are irregularly stained, the cross-striations are indistinct and the longitudinal fibrils are prominent. Inflammatory cells are not present in any part. Congestion of small vessels is noteworthy in six cases and mild in two others. Diffuse recent hemorrhage without reacting inflammatory cells is found under the endocardium and among the neighboring muscle fibers in one animal.

In frozen sections stained for fat, it is demonstrated as very minute droplets, usually distributed unequally among the fibers. In six specimens the amount is extreme, nearly all the fibers being involved; in sixteen the fatty degeneration is marked; in five it is mild; no fat is found in two; while in one case the tissue is not available for study.

Spleen: Sections of normal rhesus spleens show much lymphoid tissue with active germinal centers, a relatively small amount of pulp and almost collapsed blood spaces. In the experimental animal, the lymph nodules are regularly much reduced in size and widely separated by marked to extreme congestion. In three specimens, there are distinct zones of hemorrhage within numerous nodules.

The germinal centers are small, devoid of lymphoblasts and are composed of large, elongate stroma and endothelial cells. These remains of the germinal center, present in about half the thirty specimens, contain small numbers of lymphoblasts in seven instances.

A conspicuous finding in all but four specimens is necrosis in and about the germinal centers, which is manifested by disorganization, swollen disintegrating cells and much nuclear débris. Even in the absence of the germinal center, necrotic lymphoid cells of the nodule are often found. Constantly accompanying the necrosis, there is a response on the part of the endothelial cells, without, however, any polymorphonuclear infiltration. The endothelial cells probably have a local source and assume phagocytic activities, since one can trace such cells, marked by contained brown pigment granules, from their location in the germinal centers of the normal spleen through changes responding to the necrosis of the lymphoid cells, to their own degeneration. Normally they are flat or rounded; in experimental specimens they are often swollen, vacuolated and phagocytic; and in about one-fourth of the specimens, they show necrosis and disintegration.

Satisfactory frozen sections of this organ, stained for fat and counterstained with hematoxylin, were obtained from twenty-eight specimens. After careful search of several sections of the normal spleen, small numbers of fat granules were seen in only two or three endothelial cells of the germinal centers. In the experimental tissues, extracellular granular fat is demonstrated in nine instances in the necrotic areas of the lymph nodules. In the same areas, many endothelial cells containing fine fat granules are found in nineteen specimens and fewer such cells in an additional six tissues.

Not only is there an endothelial response within the lymph nodule, but also about the nodule and in the pulp. In half the tissues of this group of animals, there is a distinct, and in some cases marked, hypertrophy and apparent hyperplasia of the endothelial cells. These cells are large and rounded, their nuclei are commonly weakly stained and occasionally they show degenerative changes. Endothelial leucocytes (free mononuclear cells) are likewise mildly to markedly increased in numbers in about two-thirds of the specimens; they often demonstrate mitoses and rarely are phagocytic. In numerous instances these two classes of cells are especially prominent and numerous in the neighborhood of the lymph nodule.

In the frozen sections no fat is demonstrated in the endothelial cells of the sinusoids of the normal spleen, but in the experimental animals granular fat is stained in many of these cells in about one-third of the specimens.

Polymorphonuclear leucocytes are variable in occurrence. They are conspicuous in the pulp in eleven instances and are in large numbers in six additional cases. As a rule, these cells are scattered and only in one instance are also in foci.

Lymph Nodes: Sections of these structures are available for examination from twenty-two animals. In nineteen cases, they are from the axillary or inguinal regions, and in other instances from miscellaneous sources. While the lymphocytes are not appreciably reduced in numbers, other changes are similar to those in the spleen: the germinal centers are small and often devoid of lymphoblasts; necrosis in the region of the germinal centers is present in over half the specimens and accompanied by enlarged, phagocytic endothelial cells. Several sections of lymph nodules from miscellaneous sources (gastric and duodenal submucous, peribronchial, mesenteric, in the region of the pancreas) likewise show the same degenerative changes.

Phagocytic endothelial leucocytes are common in the sinuses, but are also found in the normal lymph nodes. Congestion is noteworthy in a few specimens, while hemorrhage is not encountered.

Lungs: Recent hemorrhage into small groups of alveolar spaces or in the subpleural region is present in thirteen specimens. This effusion of blood is not massive, no break in the alveolar wall is obvious and an inflammatory reaction does not result. In a few instances when cysts of *Pneumonyssus griffithi*² are in the sections, small areas of recent hemorrhage are commonly adjacent. Congestion of alveolar capillaries is not conspicuous, but is found in eight of the thirty specimens and not always coincident with the hemorrhages.

Stomach: Of the twenty-four sections of stomach available for study, only three show small areas of recent hemorrhage in the mucosa and without inflammatory reaction. In an additional three specimens, appreciable numbers of extravasated red blood cells occur in the same location. They are also found on the mucosal surface, usually not coincident with the extravasations in the mucosa, in seven instances. No lesion or erosion is evident in any case, and congestion of mucous or submucous capillaries is neither common nor extreme.

Intestines: Sections of intestine were not examined routinely and only one of the few studied shows an extensive submucosal and less subserosal hemorrhage without inflammatory reaction. No lesion in the mucosa is found.

Adrenal Glands: The outstanding pathology of these structures is necrosis of the cells of the zona fasciculata. This occurs in about half the thirty specimens of the series. The necrosis involving numerous scattered cells to small and large groups of cells, is manifested by loss of staining property, disorganization, and fusion of necrotic cells. Karyorrhexis is common and much nuclear débris is often present. Polymorphonuclears invariably react to the necrosis and are found in large numbers both in the neighboring blood spaces and within the necrotic cells or areas.

Eight specimens show congestion of the small blood vessels. In three cases there are either mild or marked recent hemorrhages in the zona fasciculata and between this layer and the medulla. Almost every specimen, as well as those from the control monkeys, has from two to six peculiar calcareous concretions in a section in the medulla close to the cortex.

Pancreas: This organ is not essentially altered aside from frequent congestion and the acute pancreatitis in one animal (No. 229).

Voluntary Muscle: The variation in staining reaction of the muscle fibers and occasional loss of cross-striations are similar to the findings in the normal rhesus tissue. Likewise, the frequent cysts of Sarcosporidia are present both in the control and experimental animals.

Brain: Six specimens of this organ demonstrate no essential deviation from normal, aside from occasional mild congestion.

DISCUSSION

The type or degree of pathology shown by these animals does not seem to depend on the method by which the virus was transmitted.

Fatty degeneration is a prominent feature in the liver, kidney, heart and spleen. In the first three of these organs, the parenchymatous cells are thus regularly affected, while in the spleen the necrotic areas in the nodules and endothelial cells of the pulp and nodules show a fatty change.

Necrosis is likewise common, involving the liver, kidney, lymph nodules of the spleen and regional nodes, and adrenal glands. Cloudy swelling is more extensive than necrosis, however, in the renal epithelium. Apparently in response to the degenerative changes, polymorphonuclear and endothelial leucocytes are usually increased in the liver, the latter type of cell responds in the lymphoid system,

polymorphonuclears react in the suprarenals and no inflammatory cells are found in the kidney or heart. It seems that these reacting cells are called out by more advanced degeneration, rather than by early degeneration or the presence of the virus, since we find them only in those structures showing marked necrosis, and neither in the kidney and heart nor scattered generally where it might be supposed the virus has been.

Hemorrhage as it occurs in the gastric mucosa, lungs and liver, is recent, without inflammatory reaction and not extensive. In view of the fact that blood effusion is mild and is often found microscopically when not seen in gross, it seems that the extravasation of blood is rather by seepage than by rhexis of a vessel. In two instances (sections of intestine and adrenal gland), however, the hemorrhages are extensive.

No definite evidence is furnished by this study as to the source of the jaundice in these animals. The bile capillaries do not contain inspissated bile and the bile ducts are not remarkably altered. There is a severe disorganization of the parenchymatous hepatic structure, but the endothelial cells of the sinusoids are relatively unaffected. It is noteworthy that although some yellowish brown pigment granules are found in the endothelial cells of the normal splenic pulp, much more pigment is regularly demonstrated in these cells in the experimental monkeys. In frozen sections, the blood spaces are often beautifully outlined by pigment granules.

In the liver parenchyma, an acidophilic, granular type of degeneration is constant and hyaline changes often take place. Nuclei of degenerating liver cells commonly undergo acidophilic changes. While strict midzonal necrosis occurs in only one specimen, the common presence of most extreme necrosis in the intermediate zone and the absence of degeneration of either other zone alone, indicate that the type is fundamentally midzonal and probably begins as such. This view is supported by the fact that the one specimen (No. 304) showing early changes, has undergone fatty degeneration only in the midzonal cells.

The acute degenerative changes of the kidney epithelium and the presence of casts in the tubules indicate the source of the abnormal urine findings of albumin and casts. The occasional presence of lime deposits are likewise of interest.

The sequence of events in the splenic nodule appears to be a dimin-

ution in the number of lymphocytes and lymphoblasts, followed by necrosis of the latter cells and neighboring lymphoid cells, sometimes with fatty changes; the whole is accompanied by a local endothelial activity, and in turn, by fatty degeneration and necrosis of the endothelial cells. Many specimens show small lymph nodules and no trace of germinal centers. While this may be due to the age of the animal, it seems rather that the centers have disappeared in the course of the disease, since at necropsy only a few monkeys were obviously old and microscopic evidence of advanced age is seldom encountered. Lymphocytes are not increased in the blood spaces and the supposition is that the lymphocytes disappearing from the lymph nodules are not diffused through the pulp. The presence of polymorphonuclears is probably in response to the degeneration of endothelial cells.

Special staining for microorganisms in the monkey tissues has been undertaken in three ways, aside from the search in the ordinary paraffin sections. These are Levaditi preparations for spirochetes and leptospiras, Giemsa stains of paraffin sections, and Giemsa, Fontana and other methods of examining smears of fresh tissue. Levaditi preparations, controlled by guinea pig tissues showing large numbers of *L. icteroides*, have not shown any such form in monkeys either running the full course of the disease or killed on the first day of fever (killed monkeys are not included in the series discussed in this paper). Giemsa stains of the paraffin sections have demonstrated bacteria, appearing as postmortem invaders, only in those animals necropsied some time after death. In a limited number of monkeys in which the disease ran a fatal course, in one monkey killed on the first day of fever, and in controls, Giemsa, Fontana and other bacterial preparations were made of fresh smears of the spleen, liver and kidney; no bacterial forms have been found that are not also in the control smears.

SUMMARY

The microscopic pathology in thirty *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of yellow fever virus is described.

In the liver, fatty degeneration, necrosis and nuclear changes are prominent. Polymorphonuclear and endothelial leucocytes are commonly present.

The renal epithelium shows fatty degeneration, cloudy swelling and less extensive necrosis. No inflammatory cells are found. Tubules contain hyaline, granular and, in a few instances, calcareous casts.

Fatty degeneration is an almost constant finding in the muscle fibers of the heart.

The pathology of the spleen includes congestion, diminution of lymphocytes and lymphoblasts, necrosis of lymph nodules and a marked endothelial response in the nodules and pulp. Fat is demonstrated in this organ in the necrotic areas and in the endothelial cells of the nodule and pulp.

Regional lymph nodes likewise show necrosis and endothelial activity.

The lungs and stomach furnish evidence of recent hemorrhage without inflammatory reaction, mild in degree and without obvious lesions in the vessel wall.

Necrosis with a polymorphonuclear reaction is common in sections of the adrenal gland. Mild to marked hemorrhage occurs infrequently.

Tissues so far studied and showing slight or insignificant changes are the brain, pancreas and voluntary muscle.

No bacteria, leptospiras or spirochetes have been demonstrated. The lesions of the various organs may be explained on the basis of a severe intoxication and no nidus of the virus is suggested.

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THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHEBUS* *

III. COMPARISON WITH THE PATHOLOGY OF YELLOW FEVER IN MAN

N. PAUL HUDSON, M.D.

The preceding papers ¹ have presented the gross and microscopic pathology in the *Macacus rhesus* fatally infected with the Asibi strain ² of yellow fever virus. The purpose of this paper is to compare these findings with those in human cases of yellow fever.

The literature regarding the pathology of yellow fever in man largely concerns this disease as it occurs in the Western Hemisphere. There are, however, a few references available on this subject from West Africa, including articles by Aitken, Connal, Gray and Smith,³ Aitken and Smith,⁴ Klotz and Simpson,^{5, 6} and notes by Stevenson,⁷ Turnbull,⁸ and Boyce.⁹

GROSS PATHOLOGY

In order to obtain more data on the gross pathology of this disease in West Africa, we reviewed the records of thirty-three cases confirmed by microscopic study, contained in the files of the West African Yellow Fever Commission. These necropsy records are available through the courtesy of the medical authorities of Nigeria and the Gold Coast, British West Africa. The postmortem examinations were made in most cases by the local medical officer or pathologist and occasionally by members of this Commission. The microscopic studies were completed by the pathologists at the Medical Research Institutes of Accra, Gold Coast, and Lagos, Nigeria. In addition, all cases but two were examined microscopically by the pathologists of this Commission with complete agreement as to the diagnosis. We wish to express our thanks and appreciation for the coöperation and interest manifested by the medical administrative officers and by individuals who conducted the postmortem examinations, submitted their records for our use and sent specimens for our examination.

* The studies and observations on which this paper is based were made with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

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Except for four Syrians and four native Africans, the patients were Europeans, at the time resident in West Africa. A brief résumé of the reported findings in these thirty-three cases follows:

Jaundice: regularly of the skin and a little less often of the sclerae; commonly of the aorta and other large vessels near the heart, cardiac valves, and subcutaneous tissue and fat; frequently of the costal cartilages, renal tissue, and heart musculature; and less often of other parts and body fluids.

Hemorrhage: as petechiae, in a third to a half of the cases, in the skin, pleurae, epicardium and endocardium, mucosa of small intestine, and beneath the renal capsule; occasionally in the peritoneum, papillary muscles of the heart, bladder mucosa and mucosa of colon; and rarely in the subconjunctival tissue, retroperitoneum and mucosa of trachea. Altered blood ("black vomitus") in the stomach in twenty-nine instances and mucosal hemorrhages in twenty-four; blood in the small intestines in twelve, with mucosal hemorrhages in fourteen; in the colon, blood in four and hemorrhages in three. Hemorrhages and congestion in the lungs in about two-thirds of the cases. Evidence of bleeding gums frequently recorded.

Liver: regularly "boxwood" in color, described as yellow, yellowish brown, yellow-khaki, or reddish yellow; sometimes enlarged; and on section, often friable, fatty, mottled by lobular markings, but seldom congested and hemorrhagic.

Spleen: usually enlarged, congested, firm and malpighian bodies often prominent; malaria in one case (a native African).

Kidneys: enlarged and congested in over half the cases; often icteric; cortex swollen, cloudy and fatty; and blood in renal pelvis in four instances.

Heart: often pale or pale brown; sometimes soft, and certain parts hemorrhagic as described under the subject of hemorrhages.

Bladder: occasionally empty but as a rule containing bile-stained urine, positive for albumin, casts and bile.

Other Organs: adrenal glands, normal; pancreas, occasionally bile-stained and congested; lymph nodes, not remarkable or slightly enlarged; brain (one specimen), mildly congested. Four patients were women; uterine hemorrhage was found in two cases, of whom one was known to be menstruating at the time.

Cutaneous jaundice and petechiae were obviously difficult to determine in the four cases of African natives. Likewise, any abnormal

coloration of the liver and fat in these individuals is confused by the pigmentation caused by the common diet of palm oil. It should be noted that the necropsy observations were made by several investigators and in some cases were incomplete. Taken as a whole, however, the above résumé with articles of Aitken and others,^{3,4} displays the fact that in the gross findings, yellow fever as it occurs in West Africa is not essentially different from yellow fever of the Western Hemisphere. It is not within the province of this paper to go into details as to the comparison of these findings in the two hemispheres, but simply to furnish a basis for comparison with the findings in the *Macacus rhesus* to which, thus far, yellow fever has been transmitted only in West Africa. The reader is referred to articles describing the pathology of yellow fever in the Western Hemisphere, by Boyce,⁹ Marchoux, Salimbeni and Simond,¹⁰ Marchoux and Simond,¹¹ Rocha-Lima,¹² and more recently by Noguchi,¹³ Elliott,¹⁴ and Muller and Blaisdell.¹⁵

COMPARISON OF GROSS PATHOLOGY OF HUMAN AND MONKEY CASES

Icterus: A constant finding at necropsies of human victims of this disease, was also manifest in the monkeys, but to a less intense degree. Thus while the skin of monkeys was not, as a rule, jaundiced and the tarsal conjunctivae not always deeply colored, a yellow or greenish yellow color of the laryngeal cartilage, large vessels near the heart and body fluids was regularly observed, and other parts were irregularly icteric. It is to be noted that the abnormal coloration was the same in man and rhesus, that is, a lemon yellow and not an orange or brown color.

Hemorrhages: These were found in both human and monkey cases in the pleurae, lungs, gastro-intestinal tract and gums, but were rarely observed in this series of animals in the endocardium and not seen in the skin, peritoneum, retroperitoneal and perinephritic tissues, epicardium, bladder, liver or kidneys. Petechiae were smaller in the animals than were commonly found in man, but in both the hemorrhages were of recent origin. Altered blood in the stomach deserves special mention because of its prominence. In the series of human cases it occurred in almost every instance, while in the monkeys it was found in one-third of the specimens and

was remarkably similar in appearance. As in man, "black vomit" was often found unassociated with mucosal hemorrhages. Likewise, the intestinal contents were colored reddish brown or black by admixed altered blood both in human cases and in monkeys, with perhaps a greater tendency to mucosal hemorrhages of the duodenum in man.

Lungs: Hemorrhagic changes were present in the lungs of the rhesus as well as of man, and the absence of congestion in the experimental lungs, when the necropsy was done immediately after death, probably indicates a difference in posture.

Liver: The color, pallor and fatty appearance of the liver were strikingly similar. The friability and lobular mottling of the liver tissue, when observed in humans, was the same as in the monkeys. Hemorrhagic areas were absent in the animal livers but there was an agreement in the usual dry, bloodless condition of the sectioned tissue. In the human, the liver was reported to be enlarged in some cases; in experimental yellow fever, we felt that the slight enlargement in some instances was within normal limits.

Spleen: The gross findings in the spleen were alike as regards enlargement, congestion and firmness. There was this difference, however: whereas in the human, the malpighian bodies were often prominent and contrasted with the surrounding congested tissue, in the monkey these follicles were usually very small and poorly outlined. It is of interest that in the records of several human necropsies, the lymph follicles were reported as indefinitely outlined and barely visible.

Kidney: Acute degenerative changes in the kidney as evidenced by an enlarged organ and a cloudy, swollen cortex were found in both classes of specimens. Congestion, often referred to in the human cases, was not a conspicuous feature in the monkey kidneys, and hemorrhage was likewise not seen in the experimental tissues. Icterus, common in the monkeys, was often recorded as being present in human kidneys.

Heart: The heart was sometimes pale and yellowish both in man and in the rhesus and the weight was similarly unchanged. As previously mentioned, the cardiac surfaces were often icteric, but the tendency to hemorrhage in these surfaces was greater in human cases.

Urine: The urinary findings were identical, but hemorrhages were not found in the bladder mucosa of monkeys.

The organs that were spared or showed minor changes were the same in human and animal cases: adrenal glands, lymph nodes, pancreas, organs of the neck, voluntary muscle and brain. Data on the condition of human genitalia are incomplete but in the monkey only jaundice of mucous surfaces was apparent.

SUMMARY

It is evident that similar pathologic processes have taken place in the organs of human and rhesus cases of yellow fever. Jaundice, hemorrhage of various parts, "black vomit," pallor, and fatty necrotic changes in the liver, acute degeneration of renal parenchyma, splenic congestion and urinary findings were present in both man and monkey. Although variation existed as to the degree of intensity or extent of involvement of parts, qualitatively the parallelism was striking as regards the icteric color, the recent hemorrhages, and the appearance of the liver, kidney and spleen.

MICROSCOPIC PATHOLOGY

The histologic pathology of yellow fever has been a subject of considerable study, with particular attention paid to the striking lesions in the liver. Councilman¹⁶ early described the hyaline bodies found in the liver cell. Rocha-Lima¹² has emphasized the midzonal location of hepatic necrosis, while Marchoux and Simond¹¹ have shown the tendency to fatty degeneration throughout the organs. Seidelin¹⁷ points out that the liver is the seat of constant pathology, in which there is variation in extent of parenchymatous involvement, but in which disorganization of tissues, necrosis and fatty degeneration are conspicuous. As regards microscopic studies of this disease occurring in West Africa, reference should be made to the articles previously cited, by Aitken and his collaborators,^{3, 4} Stevenson,⁷ Turnbull,⁸ and recently by Klotz and Simpson,^{5, 6} who have contributed especially to the study of the spleen in this disease.

We have had the opportunity of studying the tissues from thirty cases of yellow fever occurring in West Africa (Nigeria, Gold Coast, Gambia and Senegal). These specimens did not always include all the organs, but at least the organs showing the changes essential for

diagnosis were available. In addition, tissues from ten yellow fever cases occurring in the Western Hemisphere were studied in Toronto, Canada, through the kindness of Dr. Oskar Klotz. From these studies, we can concur in the opinion of Klotz and Simpson,⁵ that "no fundamental difference was to be noted in the pathology of fatal yellow fever cases of West Africa and the Americas." It is not the purpose of this paper to deal with a comparison of the pathology of this disease in the two hemispheres, but to compare the pathology of the monkey tissues with that discussed in the literature and studied by us in human cases. The reader is referred to the works of the various investigators enumerated and to an article in preparation by Klotz ("A fuller study of the comparative pathology — will be taken up in another report" ⁵) not yet at hand.

Liver: As in the human, the liver (Figs. 1 to 4) of experimental animals shows the most striking lesions. There is the same type of fatty degeneration with the finer particles of fat often in the midzone; in the animal, however, the quantity of fat stained is more than in most human cases. Likewise, a similar type of necrosis exists in the two sets of tissues, both as to being a granular acidophilic form of degeneration of the parenchymatous cells, and as to demonstrating a midzonal location. In the two sets of tissues, the extent of involvement varies from a strict midzone to inclusion of cells almost to the limiting portal area and central vein, but in no instance has there been seen either a periportal or central necrosis alone. The accompanying hyaline type of degeneration is more conspicuous and common in the human than monkey liver. Both man and rhesus livers show jumbled and irregular, necrotic and completely disintegrated cells, with loss of trabecular arrangement but no loss of cell or lobular space. Nuclear changes are similar, with probably more karyorrhexis in experimental specimens and no acidophilic granules in degenerating nuclei in human cases; there is found, however, the same small compact acidophilic nucleus in some acidophilic cells.

The endothelium of the sinusoids is preserved in the rhesus as well as in man and the Kupffer cells are similarly little changed, although more frequently swollen and phagocytic in man. Numerous specimens of monkey liver show numbers of extravasated red blood cells, and only two of the thirty livers studied demonstrate hemorrhages of appreciable degree. It is much more common to find hemorrhages as well as extravascular red blood cells in human specimens. Inflam-

matory cells, of the polymorphonuclear and mononuclear types, are found more regularly in the experimental tissues both intravascular and extravascular. Occasionally, however, they are entirely absent and, conversely, are sometimes found in human cases.

The portal areas of rhesus more than in human livers show variable numbers of lymphocytes and endothelial leucocytes, but the inconstancy of these cells prevents any significance being attached to them. Bile ducts are regularly normal and no inspissated bile is seen in bile capillaries of either set of tissues.

Spleen: The spleen (Figs. 5 to 8) presents interesting comparative pathology in that the following characteristics are similar: congestion, small lymph nodules, and an endothelial response in the nature of enlarged endothelial cells of the pulp, especially about the nodules, and an increase in free endothelial leucocytes. While in man the germinal centers are generally lacking and rarely show necrosis, in the rhesus remnants of the centers are commonly present and necrosis with large phagocytic endothelial cells is regularly seen. Polymorphonuclear leucocytes are found in the pulp in numerous experimental sections, whereas it is uncommon to find them in the other group of tissues. Klotz and Simpson⁶ and others have referred to fatty degeneration of the endothelial cells of this organ, and in the experimental animals, fat is demonstrated in these cells lining blood spaces and in degenerated phagocytic endothelial cells in necrotic lymph follicles.

Kidney: The pathology in the kidney (Figs. 9 to 16) is the same in the two classes of specimens, with differences only of degree. Thus cloudy swelling and necrosis of tubular epithelial cells are found, but more of the former and less of the latter change is seen in the monkey kidneys. Numerous acidophilic degenerating cells are common to both groups of sections. Fatty degeneration occurs in the experimental as in the human tissues, both as to involvement of the tubular epithelium and as to the sparing of other structures. However, this type of degeneration is more pronounced in the animal specimens. Both classes of tissues show a consistent absence of inflammatory cells.

Congestion of small blood vessels, particularly of the glomerular tufts, is a regular feature of human cases, but is only occasionally found in monkey tissues. Hemorrhages are not seen in either group. Tubules contain much granular debris and casts of hyaline, granular

and calcareous types, more regularly, however, in human specimens. A type of degeneration of epithelial cells seems to be at least one source of "lime casts" in monkeys, such as Muller and Blaisdell¹⁵ have described in human kidneys. Glomeruli are almost entirely unaltered, but their capsular spaces contain granular debris in specimens from man more than in those from the other group.

Heart: The heart (Figs. 17 and 18) in both sets of tissues shows fatty degeneration of finely granular form although, as in the liver and kidneys, fat is demonstrated as a rule in larger amounts in the rhesus specimens. Likewise, in both tissues, the muscle fibers are sometimes irregularly stained and cross-striations are indistinct, but these characteristics lose their significance when experimental sections are compared with those of control monkeys. Congestion and hemorrhage play a minor part in both human and monkey tissues and inflammatory cells are regularly absent.

Lungs: Sections of lungs (Figs. 19 and 20) show recent hemorrhages without inflammatory reaction, as a rule, however, more frequently and extensively in man. Similarly, congestion and edema are much more commonly found in the human tissues.

Stomach: The stomach has a greater tendency to congestion and hemorrhage in man, but there is the same type of small hemorrhages and extravasation of red blood cells without inflammatory reaction and without obvious lesions in the vessel wall.

Adrenals: Adrenal glands present contrasting pathology in that monkey sections often demonstrate necrosis accompanied by polymorphonuclear infiltration, which was found by us in one human case but is not recorded by others. Frequent congestion and an occasional hemorrhage are common to both classes of specimens.

Pancreas: The pancreas has been equally unaltered both in man and in monkey.

Lymph Nodes: Specimens of lymph nodes are not commonly available for study from human cases and little is recorded in the literature. In the rhesus, the changes are similar to those in the spleen.

Brain: The brain has been infrequently studied and shows little variation from the normal.

SUMMARY

Fatty degeneration of the liver, kidney, heart and spleen is of the same type in man and rhesus, although more extreme in the latter.

Other degenerative changes of the liver, kidney and spleen are likewise similar as to incidence, type and location. Necrosis of the adrenal glands is only rarely seen in the human, although commonly in the rhesus.

In both sets of tissues, inflammatory cells are lacking in response to hemorrhage in any organ and to the degenerative changes in the kidney and heart. Polymorphonuclear and endothelial leucocytes are usually found associated with the liver lesions in monkeys, while seldom in man.

Hemorrhages and congestion tend to be more frequent and extensive in the liver, lungs and gastric mucosa in human cases, but the hemorrhages are alike in being focal, recent and without obvious lesions of the vessels.

DISCUSSION AND CONCLUSIONS

It should be borne in mind that these papers on the gross and microscopic pathology in the *Macacus rhesus* and comparison with human pathology are based on the use of one strain of yellow fever virus. However, the pathology induced by two other strains we are studying proves to be similar to that reported and discussed in these papers.

The lesions in the *Macacus rhesus*, brought about by experimental infection with yellow fever virus, seem to result from a severe intoxication, as in the case of human yellow fever and recently expressed by Klotz and Simpson.⁶ Neither in the monkey nor in man is there any evidence of the localization of the virus. We have been unable to find either in human cases or in experimental tissues any constant bacterial form, or leptospiras or spirochetes demonstrable in Levaditi preparations.

The monkey specimens of the liver tend to confirm the fact that necrosis in the liver is essentially midzonal in type in yellow fever with less altered cells increasing toward the periphery of the involved region. Likewise, as in human cases, when the degenerative changes approach the limiting structures of the lobule, the most extreme necrosis is usually in the midzone.

Klotz and Simpson ⁶ have recorded that in the spleen there is a sequence of changes involving the lymph follicles, from early enlargement of the follicle due to hyperplasia of the endothelial elements, followed by loss of lymphocytes, to final degeneration of the endothelial cells. We have not observed in human or monkey tissues the first stage given; otherwise, a study of the rhesus sections makes it evident that the process described by these workers is probably correct. A stage of necrosis is obvious in the monkeys, but uncommonly seen in human spleens in which it is possible the stage might have been passed at the time of death. We would add, however, that in monkeys, degeneration and necrosis involves the lymphoid as well as the endothelial elements of the follicles.

The fatty degeneration of the heart muscle fibers and the same and other acute degenerative changes of the kidney in the rhesus monkey add to the evidence for the clinical manifestations of this disease in man.

If experience bears out the hope that the *M. rhesus* is regularly susceptible to the yellow fever virus, this animal will prove to be of incalculable value in the diagnosis of yellow fever in man because of the remarkably accurate reproduction of gross and microscopic lesions.

NOTE: In the photomicrographs, the lesions of yellow fever are compared as they occur in a human case and in rhesus monkeys. The human case is that of H. P., Accra, Gold Coast, diagnosed as yellow fever clinically and pathologically and from whom a strain of yellow fever virus was obtained by inoculation of a *M. rhesus* with the patient's blood. We are deeply indebted to Dr. D. Duff, Deputy Director, Medical and Sanitary Services, Dr. A. C. Paterson, Senior Medical Officer in charge of the European Hospital, and Dr. A. S. Burgess, Acting Director of the Medical Research Institute, Accra, Gold Coast, for records of this case and material for histologic study.

The sections were prepared from formalin-fixed tissues, stained with hematoxylin and eosin and, for the demonstration of fat, with scarlet red and hematoxylin.

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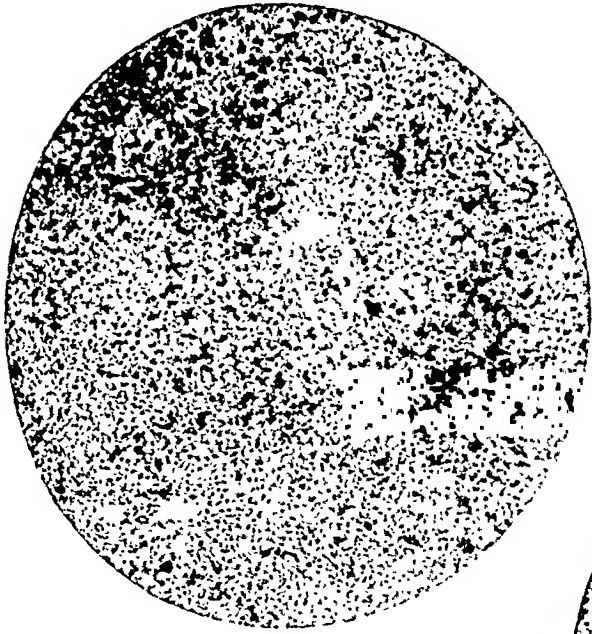
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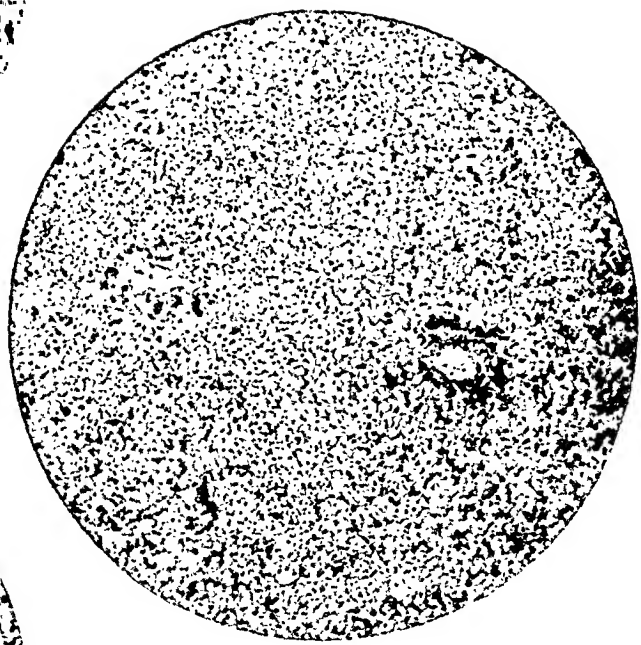
DESCRIPTION OF PLATES

PLATE 94

- FIG. 1. H. P. Liver, showing irregular band of midzonal necrosis and mild diffuse hemorrhage. $\times 55$.
- FIG. 2. *M. rhesus*, No. 312. Liver, showing fringe of intact cells about central vein (left), beyond which is zone of necrosis infiltrated with inflammatory cells. Periportal cells (right) vacuolated. $\times 55$.
- FIG. 3. H. P. Frozen section of liver, demonstrating fat; less fat in necrotic midzone. $\times 40$.
- FIG. 4. *M. rhesus*, No. 312. Frozen section of liver, showing less fat in necrotic midzone. $\times 40$.



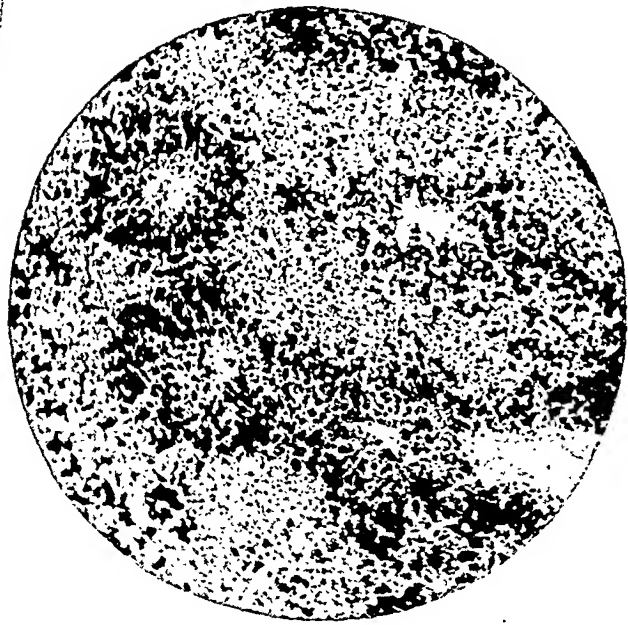
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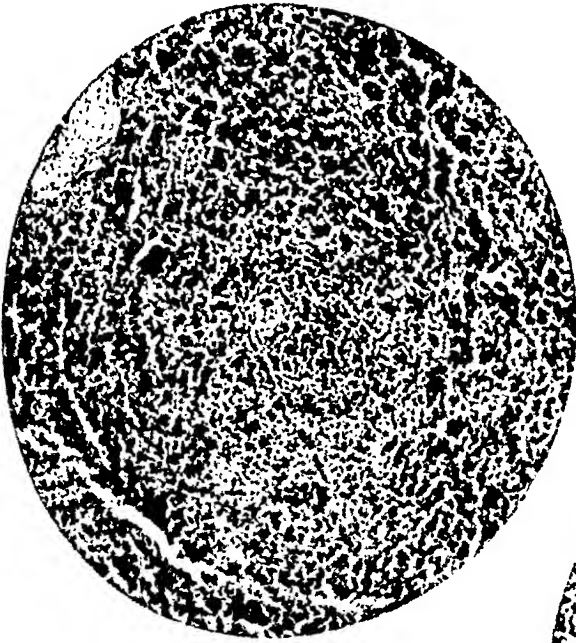
PLATE 95

FIG. 5. H. P. Spleen, showing small lymph follicle surrounded by faint zone of enlarged endothelial cells, beyond which are congested blood spaces. $\times 85$.

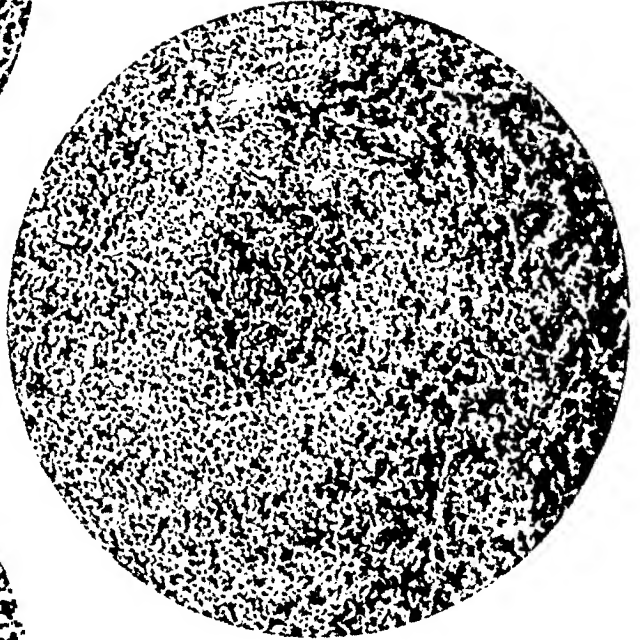
FIG. 6. *M. rhesus*, No. 344. Spleen, showing small lymph follicle surrounded by zone of enlarged endothelial cells, beyond which are congested blood spaces. $\times 85$.

FIG. 7. Spleen of normal *M. rhesus*, No. 315. (Note magnification is lower than previous pictures of spleen.) $\times 45$.

FIG. 8. *M. rhesus*, No. 362. Spleen. Necrosis in lymph follicle. $\times 150$.



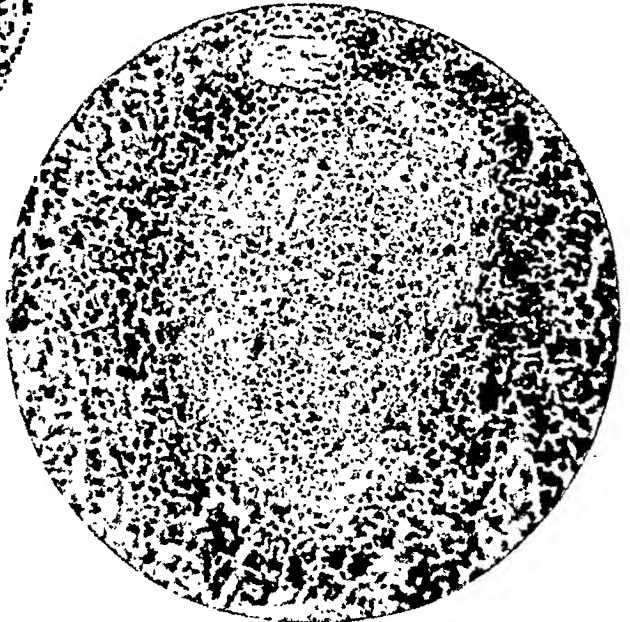
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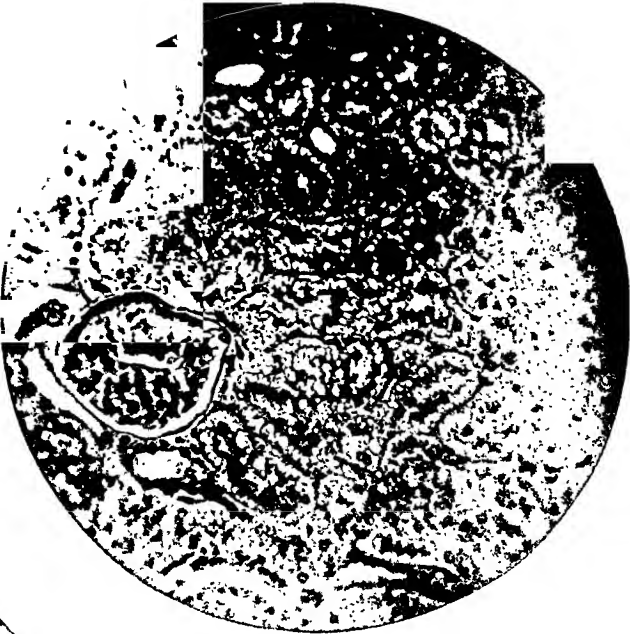
Pathology of Experimental Yellow Fever in *Macacus Rhesus*, III

PLATE 96

- FIG. 9. H. P. Kidney, illustrating swollen, acutely degenerated epithelial cells of tubules, and granular debris in lumina. $\times 150$.
- FIG. 10. *M. rhesus*, No. 327. Kidney, showing similar acute degenerative changes in vacuolated epithelial cells. $\times 150$.
- FIG. 11. H. P. Frozen section of kidney, indicating the presence of fat in tubular epithelial cells. $\times 150$.
- FIG. 12. *M. rhesus*; No. 327. Frozen section of kidney, demonstrating much fat in epithelial cells. $\times 150$.



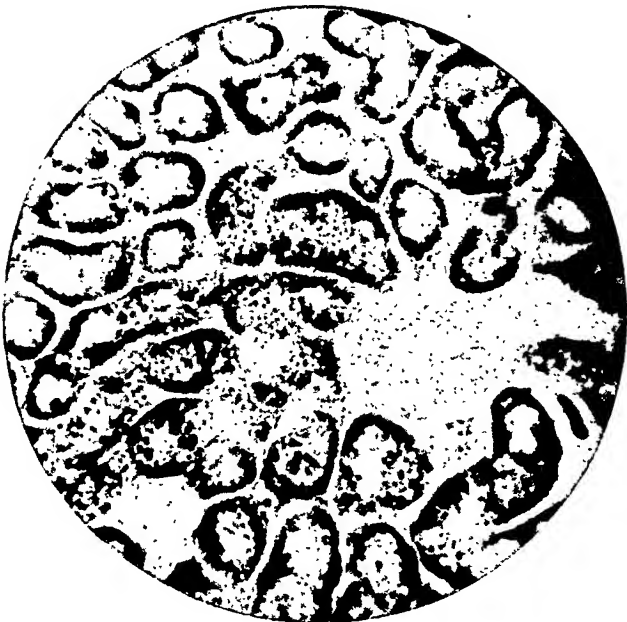
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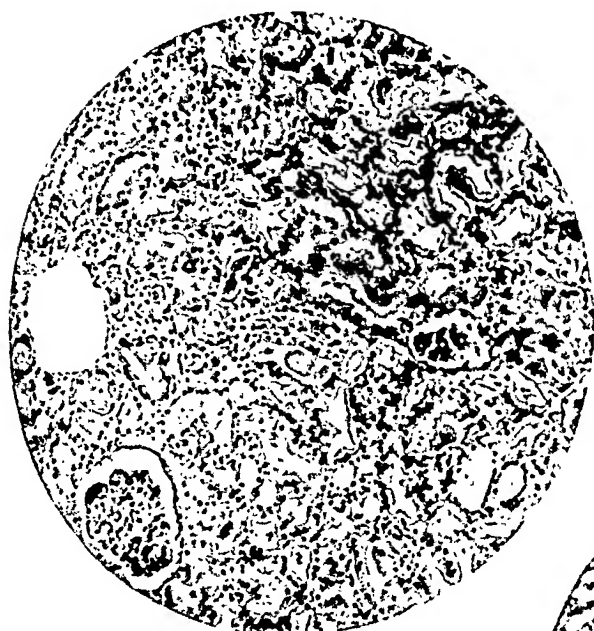
PLATE 97

FIG. 13. H. P. Kidney section, showing a few hyaline casts (center). Casts not as numerous in this case as is usually found in human kidneys. $\times 85$.

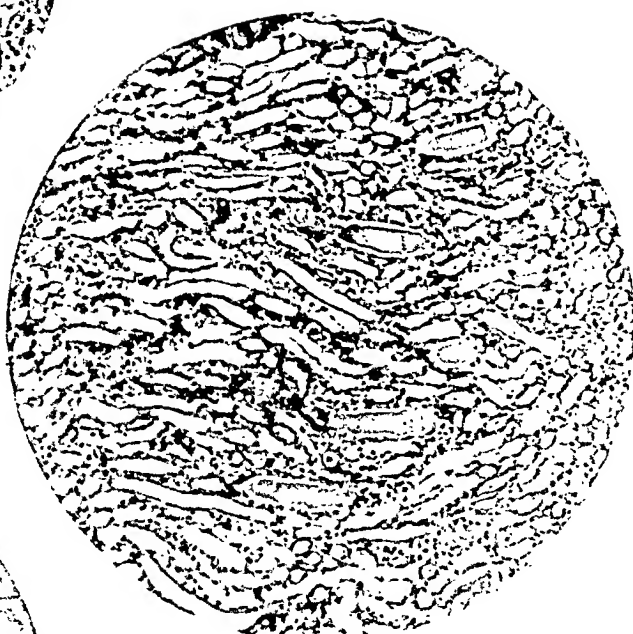
FIG. 14. *M. rhesus*, No. 346. Kidney section, demonstrating several hyaline casts. $\times 85$.

FIG. 15. H. P. Kidney, showing calcareous deposits ("lime casts") on either side of center of picture. $\times 150$.

FIG. 16. *M. rhesus*, No. 253 A. Kidney, illustrating several clumps of calcareous deposits (about center), intensely stained by hematoxylin. $\times 150$.



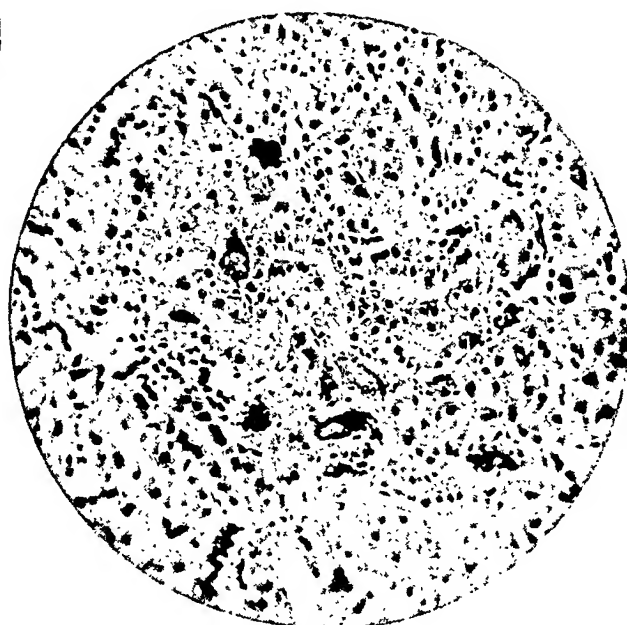
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Pathology of Experimental Yellow Fever in *Macacus Rhesus*, III

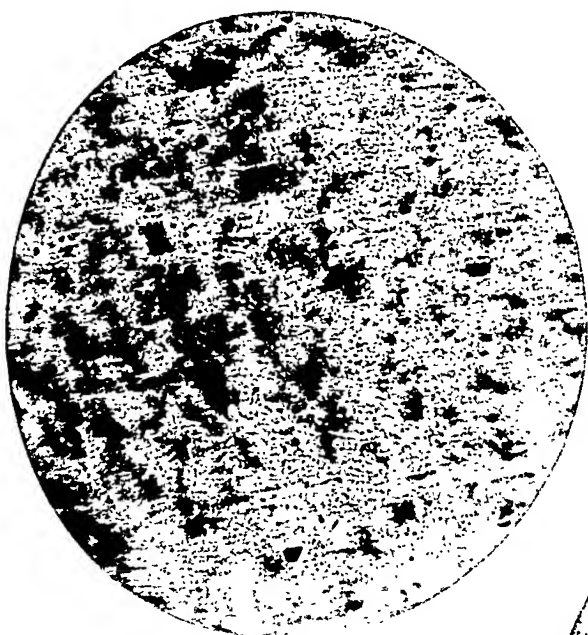
PLATE 98

FIG. 17. H. P. Frozen section of heart, demonstrating much finely granular fat in muscle fibers. $\times 150$.

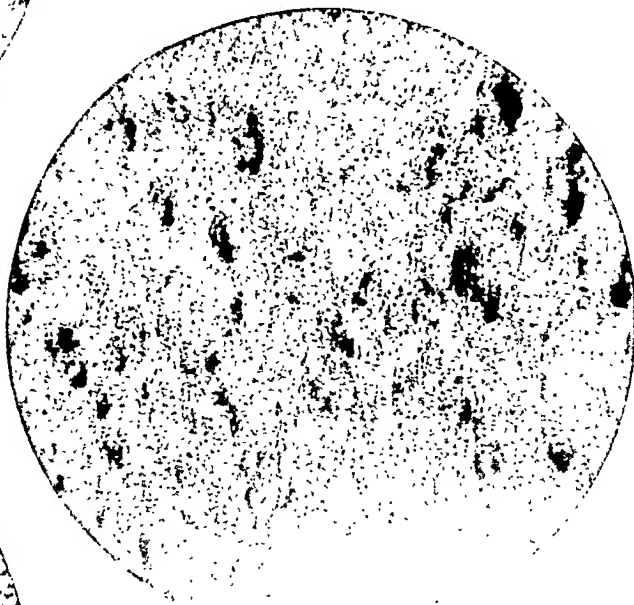
FIG. 18. *M. rhesus*, No. 327. Frozen section of heart, showing finely divided fat irregularly distributed among muscle fibers. $\times 150$.

FIG. 19. H. P. Section of lung, showing small area of recent hemorrhage into alveolar spaces. $\times 85$.

FIG. 20. *M. rhesus*, No. 316. Lung section, showing a similar recent hemorrhage into alveolar spaces. $\times 85$.



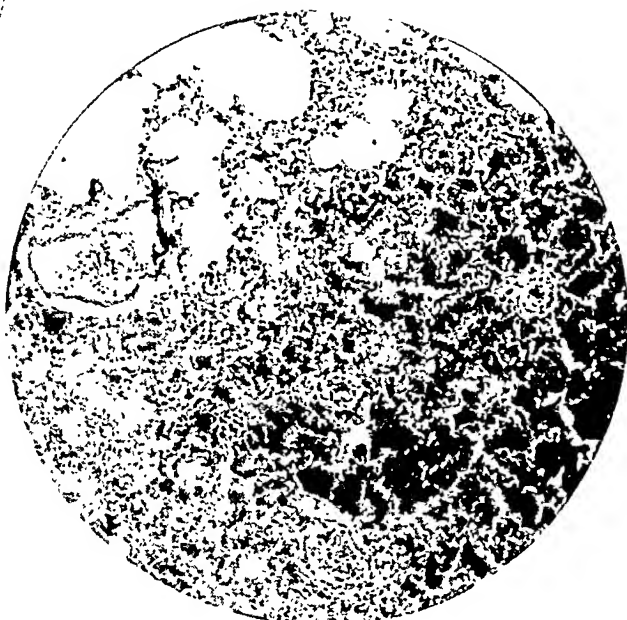
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MYOCARDIAL DEGENERATIONS IN YELLOW FEVER *

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The nature and mode of production of the toxin in yellow fever is still unexplained. Its widespread effects and influence are seen clinically and can be demonstrated pathologically, in all parts of the body. In the past particular attention has been paid to the degenerations occurring in liver, heart and kidneys. However, as yet, no satisfactory explanation has been given for the part which each of these plays in producing the death of the patient. There is ample evidence, in the clinical course of the disease, that all three of these organs are involved. Jaundice usually appears early in the course of the disease, the sclerae being jaundiced on the second or third day in many cases. The intensity of the jaundice increases with the subsidence of the fever, and may persist well into the convalescent period (Klotz,¹ Elliott,² Noguchi,³ 4th Report of the West African Yellow Fever Commission⁴). As a rule the depth of the jaundice is an indication of the severity of the disease, but in many fatal cases there is little or no jaundice (Klotz).

Uremia is considered by some authors (Elliott² and Seidelin⁵) to be the terminal event in all fatal cases. "Albuminuria appears usually on the third day; when it appears on the first day it goes to a fatal termination; on the second day it is a very bad augury." ⁴ Beeuwkes⁶ stated that "albuminuria was by far the most striking feature of this disease; in the Assamenkase epidemic of 1926, in all fatal cases, it was present in large amounts."

Elliott stated that when complete anuria occurs death usually follows in a few hours. In our own series, where histories were necessarily incomplete, one is struck with the frequency of the statement that "complete or almost complete anuria preceded death." Elliott noted the following as manifestations of uremia: air hunger, peculiar whistling respirations, precordial distress, headaches, backache, persistent vomiting, hiccough and finally delirium, Cheyne-Stokes respirations, convulsions and coma. That these are necessarily signs

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of uremia in yellow fever patients, who are already subjects of an intense toxemia and definite heart lesions, is a debatable point. The toxic effect of the disease upon the heart is made evident in three ways, by dilatation, weak soft heart sounds, and disturbances in pulse rate. The damage done to the heart is not purely transitory, nor is it limited to the acute phase of the illness; the heart is so weakened in some cases that death occurs when the patient is apparently convalescent (Seidelin). The disproportion between the height of the fever and the pulse rate (Faget's sign) is observed and reported in most accounts of the disease (Seidelin,⁷ Noguchi,⁸ Thomas,⁹ Elliott, Beeuwkes, and others). This relative bradycardia may be of such extent that a patient with a temperature of 39.5°C may have a pulse rate of 80, and during convalescence a true bradycardia of 32 to 36 beats per minute may develop.

On reviewing the reports of postmortem examinations, one finds that particular attention has been paid to the gross pathological changes found in the heart in yellow fever. The microscopic pathology of this organ has been somewhat neglected, largely because the findings were so disappointingly slight in contrast to the marked macroscopic changes seen in fresh preparations (Otto and Neumann).¹⁰ One of the best accounts of the microscopic cardiac pathology in yellow fever is given by Rocha-Lima.¹¹ He found that without exception there was more or less fatty degeneration of the myocardium. This was distributed in a somewhat patchy manner but was always more widespread than granular degeneration. On the other hand he seldom encountered myocardial hemorrhages. Nuclei were always well stained and often of strikingly large dimensions; the muscle fibrils and cross striations could always be clearly made out. Vacuolar degeneration, he stated, was well marked but interstitial processes were never noted.

Otto¹² reported varying amounts of fatty degeneration and vacuolar degeneration but no further changes in cells or nuclei. Otto and Neumann could not convince themselves of the fatty degeneration in the subendocardial and subpericardial regions as described by Sodré and Couto. They found that there was nothing remarkable in the muscle fibers. Both cells and nuclei stained well, and as in other organs evidence of inflammatory reactions were lacking.

Seidelin¹³ noted a fatty metamorphosis of the myocardium, which was frequently less marked than the gross appearance would lead

one to expect. The aortitis and endocarditis described by Sodré and Couto were regarded as evidence of secondary infection.

Noguchi stated that the muscle fibers showed one or more vacuoles situated in the central portion, suggestive of fat. Certain fibers appeared somewhat swollen. The nuclei were large and vesicular.

Marchoux and Simond¹⁴ noted that in the majority of cases the muscle fibers of the heart were slightly injured by the fatty degeneration, which may be made evident by scant traces of fatty granules throughout the length of certain fibers. On the other hand fatty degeneration may be very marked but was always limited to certain fibers beside which one saw others which were almost perfectly normal. Aitken, Connal, *et al*,¹⁵ reported that in the Lagos epidemic of 1925 the cardiac muscle showed a distinctly cloudy appearance and the fibers contained small vacuoles.

Elliott gave a detailed report of the gross and microscopic pathology observed in Guayaquil, Ecuador, in 1918. He noted that subpericardial and occasionally intramyocardial hemorrhages were found. The muscle fibers were swollen, the striations indistinct, and in the most severe cases entirely absent in places. The nuclei of the most affected fibers were absent.

The pathological findings outlined above were essentially similar in character to the degenerative lesions produced in the liver and kidneys. In the former there were great varieties in the intensity of degeneration, midzonal necrosis being a characteristic feature in typical cases. Fatty deposit was variable and was commonly found in all zones, but in mild cases may be limited to the midzonal areas, and in advanced cases may be most prominent in the portal zone. The distribution and intensity of the degeneration varied in different parts of the liver.

In the kidney, too, there was always degeneration, but the amount of change was not uniform. All stages from marked cloudy swelling to necrosis of epithelial cells were found. Casts and granular debris were seen in the renal tubules. Fatty degeneration was variable, often intensely involving all the tubules of the cortex and medulla. "In general the lesions in these three organs and throughout the body are of a degenerative type, with little or no evidence of proliferative response in fatal cases, and an absence of a primary inflammation" (Klotz¹⁶).

OUTLINE OF WORK

The material which forms the basis of this study was placed at my disposal through the kindness of Professor Oskar Klotz. It consisted of sections and blocks of material obtained at autopsy from twenty-nine cases of yellow fever which occurred in West Africa in 1925, 1926 and 1927. In addition sections were examined from ten *Macacus rhesus* monkeys, nine of which died from experimental yellow fever transmitted by direct inoculation from the blood of yellow fever patients or by bites from infected mosquitoes. The tenth monkey was killed and the sections examined as controls for the normal histology of the animal. The experimental and clinical work on these animals was done and reported upon by Drs. Stokes, Bauer and Hudson,¹⁷ and the sections made available for me by Professor Klotz. Paraffin and frozen sections were prepared and stained by hematoxylin and eosin, and with Sudan III respectively. Some sections were specially stained for fat by scharlach R. and Nile blue sulfate stains. In three cases the bundle of His was sectioned, stained and examined in the same manner as the other tissues.

MYOCARDIAL DEGENERATIONS IN YELLOW FEVER IN HUMAN CASES

Two constant features, fragmentation and granular degeneration of the myocardium, were observed in all sections stained with hematoxylin and eosin. The fragmentation of the muscle fibers was greatly increased in most cases in which the autopsy took place some hours after death. It is in part due to postmortem changes, although it may be evidence of the severity of the infection. Granular degeneration was found in all cases, but the amount and extent of it was not constant. It varied from very mild cloudy swelling in patchy areas, to involvement of large areas of the section in a marked granular degeneration. The severity of the degeneration in these latter cases, however, was not always more marked than that in which small patchy areas alone were involved. In mild cases the cross striations were not lost, but in more severe degenerations the fibers were swollen, pale staining and cross striations were lacking. In some sections curious variations in the staining of different fibers were evident. Patchy areas stained a deep pink, with a hyaline-like

appearance, were seen in some areas, whereas the greater number of the fibers stained a faint pink. Nuclear changes were seen in most of the cases examined, and varied in intensity with the amount of granular degeneration. They consisted of changes in the size, shape and staining qualities of nuclei. In cases where granular degeneration was extremely marked, nuclei had entirely disappeared. In some sections some nuclei were twice the size of others in the same area, and while some stained a deep and fairly uniform blue, others took a faint stain and appeared granular, pyknotic or vacuolated.

Small punctate hemorrhages were seen in only a few cases. They varied in size from small collections of five or six cells to clumps containing twenty to thirty red cells. In the cases mentioned the hemorrhages were not scattered diffusely throughout the section, but were limited to focal areas. The blood vessels were markedly engorged in two instances, but this was not a marked feature of the condition.

Inflammatory changes were noted in ten cases, but of these four were old pericardial lesions, and four consisted of thickenings of the walls of blood vessels together with small perivascular collections of mononuclear cells. In two cases, however, the inflammatory reaction was evidently in response to the intensity of the myocardial degeneration. In one of these, Adjei, the cellular exudate was made up of mononuclear cells, lymphocytes, plasma cells and endothelial cells in varying numbers. It was most evident where the granular degeneration was greatest, but was also seen perivascularly throughout the section. In the other case, McMillan, the reaction was limited to one small area, and consisted of focal collections of white blood cells, particularly polymorphonuclear leucocytes, in areas in which granular degeneration was most marked.

So-called vacuolar degeneration was seen in a few instances. This, however, was most evident in cases where the autopsy occurred some time after death. Consequently this vacuolation along with fragmentation is considered to be largely a matter of postmortem change and artefact.

In sections stained for fat it was always present. The extent and distribution of the fatty degeneration was markedly variable. In many cases it was not proportional to, or indicative of, the myocardial damage, as judged by granular degeneration, commonly being more extensive than the latter. In the majority of cases there was a patchy distribution of the fat throughout the fibers, the fatty

deposit being most marked about the nuclei. These fatty changes about the nuclei are not to be confused with the golden-brown granules of brown atrophy which are found particularly at the ends of nuclei. The fat was present in longitudinal rows of fine granules, throughout the length of the fibers, increasing in size to small droplets in the neighborhood of nuclei. In milder cases the fatty degeneration was seen only about the nuclei, and its patchy distribution in the section was markedly emphasized. In severe cases fatty degeneration was very marked, practically all fibers being affected, but even here the patchy distribution of the lesion was noticeable, some areas showing considerably more degeneration than others.

Unsatisfactory results were obtained in sections stained by Nile blue sulfate, since the fat appeared sometimes red, sometimes blue, and all gradations between these two colors were observed. One cannot therefore draw any conclusions from this study as to the chemical nature of the fat.

On examining sections taken from the conducting bundle the results were disappointing. In one case, Mrs. Elmore, there was a marked fatty degeneration of the bundle. In the two other cases no fatty degeneration was observed. One cannot conclude from these findings, however, that fatty degeneration of the conducting bundle was not present in more than one third of the cases. There was only a slight fatty degeneration present in sections of the myocardium from these two cases. We have found that this was not an infrequent occurrence in specimens preserved for long periods in formalin. This was particularly evident in one case where sections were stained immediately postmortem, six months later, and then again eighteen months later. The fat was markedly decreased in the second instance, while in the third set it had almost entirely disappeared. This, however, was not always the case; some specimens seemed peculiarly susceptible to the action of formalin. In so far as could be determined the formalin was the same in all cases.

SUMMARY

Fatty degeneration was observed in all cases. The distribution and intensity of the degeneration varied from field to field in the section, and from point to point in the fibers, being particularly concentrated about the nuclei. The nature of the chemical composition of the fat could not be determined by the use of Nile blue sulfate

stain. The results of sectioning the connecting bundle were inconclusive; further work should be done upon both these points.

Cloudy swelling was a constant feature, but here, too, variations in intensity were marked. Nuclear changes and fragmentation of muscle fibers were features of all cases. Hemorrhages occurred in seven cases, and in these instances were very fine punctate ones. Engorgement of blood vessels was not a marked feature, occurring only in two cases. Previous inflammatory changes were present in eight cases, while two showed inflammatory exudates which had responded to very acute degenerations of myocardial fibers.

MYOCARDIAL DEGENERATIONS IN THE MACACUS RHESUS IN YELLOW FEVER

The experimental work upon these animals was done by the late Dr. Adrian Stokes, and by Drs. Bauer and Hudson at Lagos, West Africa. A preliminary report of their work is found in the *Journal of the American Medical Association*,¹⁷ and a fuller report in the *American Journal of Tropical Medicine*.¹⁸ The first animal was inoculated directly from a human case of yellow fever, the second and third by transfer inoculations from the first, and the remainder by bites from infected mosquitoes. The clinical course of the disease was similar to that of yellow fever in man, fever occurring upon the third or fourth day following inoculation. Death resulted in from one to seven days after the initial fever. The gross pathology was similar to that of human cases, and the authors were convinced that they had successfully transmitted yellow fever to the *Macacus rhesus*.

The normal microscopic anatomy of the heart of the *Macacus rhesus* differed considerably from that of the human. The myocardial fibers were narrow and closely compacted, cross striations were well marked and easily made out. The nuclei were variable in size and shape but were usually rounded and oval in form, and large in comparison with the size of the fibers. The walls of the blood vessels were thickened. There was also an increased amount of connective tissue with a slight exudate of lymphocytes and endothelial cells about them indicative of previous inflammatory changes. Autopsies were performed immediately after death, the animals either dying of the disease or being killed by a whiff of chloroform while in a moribund state.

Sections were made from paraffin and frozen blocks of tissue. These were stained routinely by hematoxylin and eosin, and Sudan III respectively.

In contrast to human cases fragmentation was not a marked feature. However, cloudy swelling and granular degeneration were present. They varied in amount and distribution, but in severe cases cross striations were obliterated. In some sections there were patchy areas which stained markedly acidophilic, the muscle substances appearing very much like hyaline material. Nuclear changes were present in most cases. The extent of these changes, however, was not so marked as in human cases, variability in the size and staining of the nuclei was observed in normal hearts. Occasional punctate hemorrhages were seen. Inflammatory changes, aside from those seen in the normal heart, were not observed, and vacuolar degeneration was missing.

Fatty degeneration was present in all the cases studied. Its extent was variable and ranged from that seen in one case, in which only a minute quantity was present about the nuclei, to that in others in which the whole section was heavily loaded with fat. The patchy distribution of the degeneration was quite marked. The fat was found more particularly in the region of the nuclei. In instances where it was distributed throughout the fiber it did not have the regular linear arrangement of granules seen in human cases. The fat appeared in the form of fine granules, becoming somewhat more globular about the nuclei.

SUMMARY

Fatty degeneration was a constant feature varying in severity from very slight fatty change to a marked general degeneration. The patchy distribution of the lesion was seen characteristically whether the case was severe or light. Cloudy swelling and granular degeneration were constantly present, but here, too, variations in the intensity of degeneration, and patchy distribution of lesions, were observed. Nuclear changes were present in most cases. Hemorrhages were not a marked feature of the condition, occurring only in two cases. Capillaries containing red blood cells were evident in large numbers in two cases. Inflammatory changes were seen in seven cases, but these were not in relation to the disease and were

not more marked than in the normal animal. Patchy areas of hyaline-like degeneration were seen in three cases. Fragmentation and so-called vacuolar degeneration were absent in these cases.

COMPARISON OF FINDINGS IN MACACUS RHEBUS AND HUMAN CASES

The pathological findings in the heart were essentially the same in the human and in the *Macacus rhesus*. Cloudy swelling, granular degeneration, fatty degeneration and nuclear changes were common to both. The intensity of the fatty degeneration was somewhat less marked in the *Macacus rhesus*, whereas its patchy distribution was intensified. The distribution and arrangement of the fat was not so regular here as in human cases, but was similar in that it was most marked about the nuclei. Hyaline-like degeneration was a feature of the changes in the myocardium of the monkeys which seemed to be more marked than in human cases. In no case was the degeneration sufficiently intense to produce an active response of leucocytes such as was seen in two human cases. Fragmentation and vacuolar degeneration were absent in the monkeys. This further strengthens the view that they were largely due to postmortem changes, rather than results of the yellow fever, since these animals were autopsied immediately after death.

Owing to the difficulties encountered in obtaining histories and the late period at which natives seek medical aid, the clinical data in many of these cases are necessarily somewhat incomplete. Eighteen cases occurred in whites, eleven in negroes. Twenty-four cases were males, five females. The average age was 32, the youngest 4 and the oldest 57 years. Two cases occurred in children aged 4 and 5 years respectively. The onset in most cases was sudden and severe, with chills, fever, nausea and vomiting, headaches and pain in the loins. The clinical course of the disease was usually rapid, death occurring in four or five days in the majority of cases, the longest being nine days and the shortest two. Fever, black vomit, melena, anuria, jaundice and slow pulse were outstanding in the clinical signs. Jaundice was usually not very marked before death, but at autopsy after the congestion of the skin had subsided it was made out fairly well. However, in a surprisingly large number of cases, sixteen to be exact, it was noted as being very slight, while in some five cases, only mild

or moderate scleral jaundice was noted. Fever was not excessively high in any case, ranging from 100° F to 102° F in most cases and reaching a maximum of 104° F in one case. Faget's sign, or the disproportion between the pulse rate and temperature, was evident in all cases where these data were taken. This was in keeping with the findings of other observers. The causation of this slow pulse rate has been generally explained by the presence of bile salts in the blood stream. King and Stewart¹⁹ noted that the amount of bile salts in a dose of pig's bile lethal for dogs, if injected alone, will produce neither fall in blood pressure nor slowing in rate. However, they found that the amount of bile pigment contained in a dose of pig's bile lethal for dogs will if injected alone cause death, with slowing of the heart and falling blood pressure. They believed the bradycardia to be a direct result of heightened vagus tone (produced by the action of bile pigments) as atropine restored the rate. They also observed a delay in conduction time between the auricle and ventricle amounting to 2/100 to 5/100 seconds.

More recently, doubt has been cast upon the relation of jaundice and bradycardia. McVicar and Fitts²⁰ have stated that "bradycardia in jaundice has in our experience proved almost a myth; when it has been observed it has given no clue to the diagnosis." An editorial of the *Journal of the American Medical Association*²¹ stated that bradycardia was an infrequent accompaniment of jaundice, except in the intrahepatic type or the so-called acute catarrhal jaundice. Clinically in three cases that have come to our own attention recently, where jaundice and fever had been present, slow pulse was not observed. The first case was that of a young male aged 24 with an unexplained jaundice which was marked in the sclera, palms of the hands and soles of the feet. The temperature was 100° F to 101° F and persisted for five days, the pulse rate varied from 90 to 110 during the fever and subsided with it to the normal rate of 74 to 80. The second case was that of a woman dying of eclampsia with marked jaundice, some fever and a pulse rate of 110 to 120. The last case was that of a young woman aged 28, with subacute yellow atrophy; jaundice was intense (duration seven to ten days), temperature 100° F to 101° F and pulse rate 134. As a consequence of these findings, and the fact that in many fatal cases of yellow fever jaundice was not marked, one must conclude that the slow pulse rate here was not due to jaundice. The finding of marked

fatty degeneration in the bundle of His in one case was suggestive and may be a possible explanation of the slow pulse in yellow fever. Further clinical and pathological studies should be done in order to confirm or disprove this.

The findings in the above series of cases were, in general, in harmony with those previously reported. The presence of inflammatory cells in the heart in response to acute degeneration has not been noted before, although it has been reported in the liver by Klotz. The intensity of the myocardial degenerations occurring in the heart in yellow fever was in itself sufficient to account for death in some cases; but in others it must be taken as only contributory in producing death, which was induced by the general toxemic effect of the disease upon the whole body, particularly heart, liver and kidneys. The pathological findings in the heart were not sufficient to make a diagnosis of yellow fever, but taken together with the changes observed in liver and kidney are satisfactory evidence upon which the diagnosis may be made. The observations recorded in the hearts of *Macacus rhesus* were quite similar to those seen in human cases. These findings taken in conjunction with those in other organs make it apparent that a susceptible animal has been found for yellow fever. Noguchi²² reported that the *Macacus rhesus* was resistant to leptospira icteroides strains isolated from yellow fever cases in Guayaquil, while marmosets succumbed to the infection with pronounced symptoms. Their findings in these animals were different from those in our report. In hematoxylin and eosin sections the striations were visible but slightly less distinct than normal. The muscle fibers were the seat of numerous very minute vacuoles. Nuclei were normal and no hemorrhages or other forms of degeneration were present. In scharlach R. sections very fine fat droplets were sprinkled uniformly throughout the entire length of all the muscle fibers. There was no accumulation of fat about the nuclei. Similarly Muller²³ reported that there were no hemorrhages or any form of degeneration such as Zenker's. In scharlach R. sections (one monkey alone was examined) numerous fine fat droplets were sprinkled uniformly throughout the entire length of all muscle fibers. The fact that these findings were at variance not only with those in human cases, but also with those found experimentally in our series of *Macacus rhesus* suggests that the infection under these different conditions was not the same.

SUMMARY AND CONCLUSIONS

1. The microscopic examination and analysis of the hearts of twenty-nine cases of West African yellow fever, and those of nine monkeys experimentally infected with West African yellow fever, is here reported.

2. Cloudy swelling, granular and fatty degeneration were found constantly in the hearts of both the human cases and those experimentally induced in the *Macacus rhesus*.

3. Primary inflammatory changes were not seen in the heart in yellow fever. Secondary response of white blood cells to intense degeneration was observed in two human cases.

4. The distribution and intensity of granular and fatty degeneration was patchy and variable in both human cases and *Macacus rhesus*. Fatty degeneration was most marked in the neighborhood of nuclei of the fibers.

5. The causation of the slow pulse in yellow fever is still uncertain, and doubt is thrown upon the belief that it is due to the jaundice.

6. Further investigation of the clinical function and the pathological changes in the bundle of His may lead to solution of the problem.

7. The lesions in human hearts and in those of the *Macacus rhesus* are essentially the same.

8. The lesions in the heart are in themselves not sufficient to make a diagnosis of yellow fever.

In conclusion I wish to thank Professor Oskar Klotz, at whose suggestion this work was undertaken, and who has greatly assisted me during its prosecution by advice and direction.

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CORPORA LIBERA IN THE TUNICA VAGINALIS TESTIS *

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In dissecting room cadavers one not infrequently observes calcareous areas in the visceral tunica vaginalis and the albuginea. Since most of the people whose bodies are dissected here were in the later decades of life — fifty to eighty years — the processes responsible for the condition long have ceased to be active, and one is left to rely on inference. Although I do not recall ever having seen adhesions between the parietal and the visceral portions of the tunica vaginalis in these calcified regions, as mentioned by Reclus,¹ the areas apparently have a traumatic and inflammatory origin. One frequently meets with adhesions, but for some reason I have not observed calcification in them, although calcification in cases of *concretio pericardii* is not so rare. Nor have I seen ossified plaques such as that described by Cohn.²

Most of the calcareous areas which I have seen occurred in the visceral portion of the tunica vaginalis only. They were small, though some measured 6 by 10 mm. The plaques usually were scale-like, rather irregular in form, and the calcification never extended far into the depth, seemingly being confined mainly to the two tunics — the visceral vaginalis and the albuginea. The testicles upon which the calcareous areas were found seemed grossly normal otherwise. If inflammatory processes are factors in the genesis of these plaques, one might assume that both the parietal and visceral portions of the tunica vaginalis would be similarly affected, but it is possible that the difference can be attributed to the fact that the fibrous layer of the parietal portion is relatively loose, while the extremely thin visceral portion overlies the densely fibrous and relatively thick albuginea. Another reason may lie in the fact that the inflammatory processes concerned probably take their origin from within the testis or epididymis. Park³ gave a good summary regarding this condition and quoted from a number of older writers. Keyes,⁴ who gave a skiagram of a case of very extensive calcification in the tunica vaginalis, says that he "twice met with calcification of the vaginalis, a very rare condition, which was exhaustively described by Roswell

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Park." It seems strange that Keyes saw calcification only twice in an apparently large clinical experience, for it is not at all uncommon in dissecting room cadavers.

Probably more common than the occurrence of calcified areas on the testis, is the presence of free bodies in the cavity of the tunica vaginalis. I have not kept accurate count of all the cases I have seen, but recall approximately a dozen with free bodies. It is highly probable, however, that many cases were overlooked until I began to direct my attention particularly to the condition. These free bodies varied from tiny granules to about 4 mm. in diameter, and they usually were somewhat flattened, though sometimes quite spherical. A few were found lying in slight depressions on the epididymis, and one was similarly placed below the inferior pole of the testis. Some lay in the sinus epididymis, which, when well formed, is a very convenient receptacle. Most of them lay free in the cavity of the tunica vaginalis and made no particular imprint on the testis because they were small or flattened. I have never found more than four in one cavity, but have just recently found the seven shown in Fig. 1 in four out of twelve bodies, the tunicae vaginales of all of which were carefully opened and scrutinized.

Some of the larger bodies did not feel very firm to the touch, but others, even of the smaller, were so hard that they rebounded as they were dropped into a small glass vial. The testis represented in Fig. 2 contains three of the larger of these free bodies and an additional one probably lay in the depression evident on the caput epididymis. Unless students are cautioned in advance, and the greatest care is used in opening the tunica vaginalis, not only the small but also the large free bodies drop out and escape attention, and from recent observations I feel quite convinced that they are present far more commonly than I had surmised. They may be quite well known, but a fairly comprehensive search of handbooks and text books on pathology and surgery, both general and genito-urinary, both past and recent, has provided me with but a few references.

In his comprehensive treatise on pathological anatomy, which appeared in four fine royal folio volumes, two of which are composed of exquisite plates, many of which are in color, Lebert ⁵ says that Luschka thought that cartilaginous bodies of the tunica vaginalis arose from the fringes and appendices of the tunica vaginalis. Lebert held that these bodies are neither cartilaginous nor osseous,

but fibromata with calcareous centers, and he quoted Duplay (reference not cited) as saying that he had seen small cartilaginous or osseous tumors which were attached to the albuginea in the region below the caput epididymis and floating free in hydrocele fluid.

According to Duplay, these bodies vary in size from a hemp seed to a cherry, and in the quotation given by Lebert the former states that he saw small free, cartilaginous bodies with a calcareous center in four cases of hydrocele. In one of these a similar small body was still attached by a filiform pedicle in the region below the head of the epididymis. Duplay wrote that these free bodies arose from cysts which he thought might form anywhere on the testicle and later become detached. He believed that irritation of the serosa by such detached bodies would cause hypersecretion and account for the commonness of hydrocele in later years.

In discussing chronic vaginitis testis, Koenig⁶ stated that calcification occasionally occurs upon the testis in this condition, and then added that it is particularly in these cases one frequently finds pedunculated or free, firm bodies in the tunica vaginalis.

I am indebted to Dr. L. A. Sigurdson for calling my attention to a statement by Keyes upon this matter. Under the head of "Fibrous Bodies," Keyes wrote: "The so-called fibrous bodies occasionally met with upon opening a hydrocele are concretions of earthy phosphates or carbonates covered with fibrin. Probably they are for the most part due to a deposition of the hydrocele salts upon some warty growth, followed by atrophy of the little nucleus, after which the concretion breaks free. Wendlung met with concretions six times in 109 operations (Pénaire). They do not exceed the size of a pea — though Chassaignac found one 2 cm. long and 12 mm. wide — and are usually single."

The mode of formation which Keyes suggests for these fibrous bodies in hydrocele, if correct, implies that they belong to a different group than those here considered. I never have seen "warty growths" on the testis and do not know what is meant thereby.

Dr. Sigurdson further called my attention to the fact that Duckworth⁷ mentioned the presence of a calculus in a eunuchoid cadaver. Unfortunately one cannot be certain regarding Duckworth's description, for when speaking of the testes of this cadaver he merely says "in each case a hydatid body is present, and at the base of one testis

an extremely hard calcified nodule of the size of a grape stone was discovered." Duckworth unfortunately did not state that this calculus was free in the cavity of the tunica vaginalis, but if it was it may belong to the group here considered.

Péraire⁸ stated that free bodies are rare and had scarcely been described, but added "now that resection of the tunica vaginalis is performed for the radical cure of hydrocele, they will no doubt be found more frequently." Péraire apparently overlooked the fact that his fellow-countryman, Reclus, had emphasized that they are not rare, as Damaschino⁹ had stated. Reclus found them in fourteen out of 260 cavities of the tunica vaginalis. He stated that they frequently are the size of a grain of millet or of hemp, and added that their structure has long been known and also their mode of formation. According to Reclus, they are fibrous and one can observe their mode of formation on the same testicle. He stated that at first a little prominence appears which looks like a whitish spot, (*point laiteux*). Later a pedicle is said to form and when this breaks the body becomes free. He stated that three or four bodies as large as a grain of sand may be found on each hydatid; and also that pendulous free bodies are sometimes found embedded which can be expressed by force through a narrow opening or by breaking the overlying membrane. He thought that they were never found except in cases of hydrocele, and said that Mallassez found free bodies in which blood resulting from hemorrhage was still present.

It is not improbable that Reclus's statement is based on the description and mode of origin of these free bodies given by Virchow,¹⁰ according to whom proliferation may occur on the testis or epididymis without the presence of hydrocele. Virchow spoke of warty outgrowths or excrescences which were flat and lobulated or pedunculated and had a tendency to become thick and spherical at the ends. According to him, calcification occurs early and they may become larger through the addition of concentric layers, and may have the form of papillae and villi, which may be branched. He stated that they become cartilaginous in consistency and are more common in moderate grades of hydrocele and rarest in severe cases. He found them easily palpable and stated that on section he found an outer partly cartilaginous layer and an inner calcific nucleus. In free bodies as large as a cherry stone the calcific center was said to be as large as the cherry kernel. Virchow further stated that the places on

the testicle where the pedicles were attached were indicated by prominences or depressions. He found the bodies to vary in size from the head of a pin to the "bullet of a gun," or cherry stone apparently.

Soon after the publication of Virchow's lectures, Damaschino reported three free bodies found by Legroux through "a happy chance," as he says. The largest of these was 12 mm. in size and almond-shaped. All three were said to have been ivory in color, smooth, elastic and looked like fibrocartilage with a calcified nucleus. One of them apparently was bosselated.

Damaschino recognized two methods of formation, but stated that he was unable to account for the formation of some of them. According to Damaschino, Hunter and Velpeau believed that they arose from blood clots which underwent a fibrocartilaginous transformation, and which, once begun, continued even after the bodies were detached. Damaschino found some of them to contain true cartilage cells and smooth muscle, but this statement probably rests on impressions obtained from naked eye, not from microscopic examination, and belongs in the same category as the suggestion that bodies which exist detached in the tunica vaginalis may continue to grow through their *vic propre*, as Damaschino believed.

In a chapter entitled "On the Formation of Cartilaginous Bodies in the Tunica Vaginalis," Cooper¹¹ stated that they occasionally occur in cases of hydrocele. According to Cooper, they may still hang from some portion of the membrane and appear to be wholly cartilaginous, although they are that only on the outside, the center being "earthy." Cooper saw them first in the course of dissection in a case of hydrocele, and one of the illustrations accompanying his chapter represents a testis with calcification in the visceral tunica vaginalis, and very small bodies with relatively long, thin pedicles attached to the head of the epididymis. Another illustration represents a testis with the usual appendix testis and epididymis; and a third illustration represents a testis with a thick, calcified plaque in the visceral tunica vaginalis. Cooper apparently was misled regarding the structure of these free bodies by their outward appearance and consistency, and believed that they arose (1) from pedunculated bodies attached to the walls, and that they were covered by reflected portions of the membrane; and (2) from cysts which were said to occur between the visceral tunica vaginalis and the albu-

ginea. According to Cooper, these free bodies cause chronic inflammatory changes in the tunica vaginalis.

The statement of Keyes seems to be based largely on Péraire. The calculi reported on by Péraire occurred in cases of hydrocele of a half to two and a half years standing and were discovered accidentally by palpation after incision of the tunica vaginalis. They varied from a lentil to a pea in size and were said to have a watery organic outer layer which did not dissolve in hydrochloric acid. Yet it was said not to be "organized" and was found to contain "azote" and "globulo-fibrine." Double calcium magnesium phosphate was present, but iron and calcium oxalate and cholesterin were absent. The murexid test was negative.

Most of the free bodies which I have examined so far were practically or wholly calcified, as illustrated by the skiagrams in Fig. 3. Fig. 4, which is a skiagram of the entire testis shown in Fig. 2, indicates that calcification was relatively slight in the three bodies associated with it, although they were among the largest encountered so far. It is interesting that some of the smallest free bodies were found to be the hardest, although a good deal of fibrous tissue was still contained in all of them. Some were sufficiently mineralized to throw full-sized shadows, and it would seem that these should show in skiagrams of the testes taken in the living. The larger encountered by me should also be palpable in the living and might easily give rise to mistaken interpretations in skiagrams of the testis.

A microscopic examination of ten free bodies showed that some are fibrous in nature and others locular. (See Figs. 8, 9 and 10.) As shown in Figs. 5 to 9, inclusive, the interior usually is thoroughly calcified, even when the outer portion is composed of dense hyaline, degenerated connective tissue. In some cases the outer layer was partly calcified and threw as dense a shadow as the rest. Wherever the calcification was the completest, the tissue was most degenerate. None of the bodies contained a cavity lined by epithelium. As the appearance of the section of a calculus shown in Fig. 7 suggests, this might have been sessile and have become detached from the body of the testis after it was calcified, although the fibrous outer layer might also have been torn in the handling.

Although most of them have such a structure as just indicated and represented, other forms such as represented in Figs. 8, 9 and 10 also are encountered. In these the calculi are contained in locules,

each of which is formed by a framework of connective tissue. Such a structure as that represented in Fig. 10 suggests that the free body had its origin in one containing a group of tubules as contained in the appendix testis shown in Fig. 11. The epithelium of the tubules and the contents apparently degenerated and became calcified, the connective tissue septa, although also degenerate, being preserved longer. Such calculi as those shown in section in Figs. 8, 9 and 10 suggest that the larger calcified areas found in other free bodies may have resulted from the fusion of similar smaller calculi and a repetition of this process could result in the formation of a single calcified area contained in a connective tissue capsule. However, if this were the only method of formation, it would be difficult, if not impossible, to account for free bodies with such thick connective tissue capsules as that represented in Fig. 5.

In considering the origin of these bodies, the appendices of the testis and epididymis naturally first came to mind. They seemed the most likely source, although calcification of coagulum also was considered until the microscopic examination seemed to make such an origin unlikely. Since one rarely finds small cysts containing clear, watery fluid in the epididymis and the testis, it is evident that calcification of their contents might also be a source of free calculi. With degeneration of the overlying tunics they could become discharged into the cavity of the tunica vaginalis, but it would seem that a depression should then be found upon the testis in the place where they had formed, but this never was the case. Although small phleboliths are relatively common in the pampiniform veins opposite the testis, it is difficult to see how they could gain entrance to the cavity of the tunica vaginalis.

Since calcification is so common in the human yolk sac of full-term placentae, I expected to find the attached appendices of the epididymis and testis calcified with equal frequency, but I do not remember having found a single case in which the calcification was plainly evident by palpation of the appendices. Microscopic examination nevertheless showed that calcification is common. Many of the appendices, especially those on the testis, are extremely small, and since they sometimes are multiple, the presence of calcification in them made such an origin of the free bodies much more likely. One obstacle, however, is the difference in shape between the free bodies and these appendices. The testicular appendages usually are

ovoid, flattened and extremely thin, and those of the epididymis usually much larger, very soft and not infrequently pyramidal. Moreover, appendices with a thin, long pedicle are relatively rare, and it is difficult to see how sessile appendages can become detached. Nevertheless, a histologic study of testicular and epididymal appendices gives support to the inference that they are the source of the free bodies described above.

Six of the appendices testis and six of the appendices epididymis examined, were vesicular and contained calcareous contents; and fifteen and sixteen respectively were fibrous throughout, or almost so. Most of the vesicular or true hydatid type of appendices were composed of a thin, fibrous wall lined by columnar epithelium surrounding a relatively large cavity containing calcareous material, as shown in Fig. 12. The fibrous appendages, on the other hand, were usually composed of loose, relatively vascular, fibrous connective tissue, which often was surrounded by a fairly well preserved, high cubical mesothelium. Some of these fibrous appendages were decidedly plicated, as shown in Fig. 13, giving the impression in cross-section that they contained a number of cavities lined by cubical epithelium. Others were composed partly or almost wholly of a series of tubules, as shown in Fig. 11, and it is probable that the examination of a still larger series would reveal others with such a structure.

Unless the vesicular appendages, such as that shown in Fig. 12, obtained a very much thicker fibrous capsule before calcification begins, it is difficult to see how they could give rise to free bodies with a structure such as represented in Fig. 5; and, although such fibrous appendages as represented in Fig. 14 may become calcified from within, it is not so easy to see how they could form free bodies with a structure such as that shown in Fig. 5. It is significant, however, that I found one testicular appendage only about 1 mm. in diameter which had practically this structure, and this made such an origin of these free bodies very probable. Since some of the appendages examined were composed of fat, it is possible that some of the free bodies arise from degenerate, calcified, small lipomata, and others from small pedunculated fibromata, although I do not exclude such an origin as postulated by Virchow.

Although it has been suggested in connection with calcified free bodies in the peritoneal cavity that they may become encapsulated

after they have become detached, no one, so far as I know, has brought forward adequate proof to this effect. I am not familiar with the exact cellular contents of the serous fluid in the tunica vaginalis, but it is highly probable that it is similar to that of the peritoneal cavity. Since the conditions for tissue culture would seem to be ideal, it is possible, although not probable, that detached mesothelial cells may revert to a fibroblast stage and, becoming attached to a small calculus or clot, lead to its encapsulation. Moreover, if connective tissue fibers can arise from fibrin, as Baitsell¹² claimed on experimental grounds, the idea of Damaschino that they grow *par leur vie propre* after they are detached, seems less strange, although a source for the cells would still have to be found.

Although I have not examined a large series of free bodies from the peritoneal cavity, I have seen some bodies, both calcified and uncalcified, but they look quite dissimilar to the free bodies found so far in the tunica vaginalis. This statement is based on only three free bodies in the omental bursa and a few small calculi from the rest of the peritoneal cavity, however. Some of the former measured over 1 cm. in length and had a thick, fibrous wall surrounding yellowish, friable contents which apparently were partly calcified. I briefly considered their origin in 1915,¹³ and I never have found any evidence suggesting the transformation of free coagulum into connective tissue or the continued growth of free bodies in the serous cavities. Hence, without precluding another origin, I have no reason to assume it until I find free bodies in the tunica vaginalis which have a structure unlike that of appendices testis and epididymis.

It is probable that pedunculated appendices of the testis or epididymis, which do not possess an adequate blood supply, can undergo degeneration and become detached. Since the pedicles sometimes are very fine and relatively long, it is also possible that torsion may be a factor. Some pedicles contain relatively large blood vessels, but others are quite avascular. Since the latter would be dependent upon absorption for nutritive material, degenerative changes would probably appear early.

The fact that one or more free bodies may be present, even in cases in which the appendix testis and epididymis still are attached, further suggests that these appendices often must be multiple. An examination of a sufficiently large number of fetuses and newborn should confirm this assumption.

It does not seem probable that inflammatory changes play a rôle in the detachment of appendices or that such changes can play a part in the formation of the free bodies here described, unless they excite proliferative changes, as stated by Virchow. Since many of the free bodies are so very small, such an origin as this would seem to be precluded in their case, and those as large as a centimeter, reported by others, probably have both a different origin and structure than those here reported.

NOTE: The poor preservation indicated in the cross-sections is due to the fact that all this material was removed from dissecting room cadavers approximately a year after death and preservation. The mesothelium apparently is desquamated before the appendices become thoroughly calcified, and hence is not evident in any of the cross-sections of the free bodies represented in Figs. 5 to 10, inclusive.

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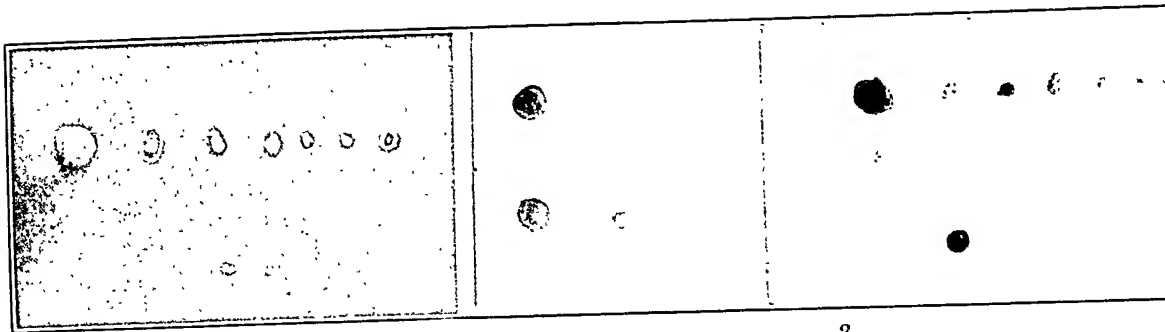
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DESCRIPTION OF PLATES

PLATE 99

- FIG. 1. Photograph of seven free, calcified bodies found in the cavity of the tunica vaginalis testis. The second and last were so dark in color that they show but faintly in the white circles. Natural size.
- FIG. 2. Photograph of testis with three free bodies. One probably was lost.
- FIG. 3. Skiagram of eleven free bodies, all of which gave almost full-sized shadows.
- FIG. 4. Skiagram of the testis shown in Fig. 2, showing that the free bodies contained only small calcified areas.

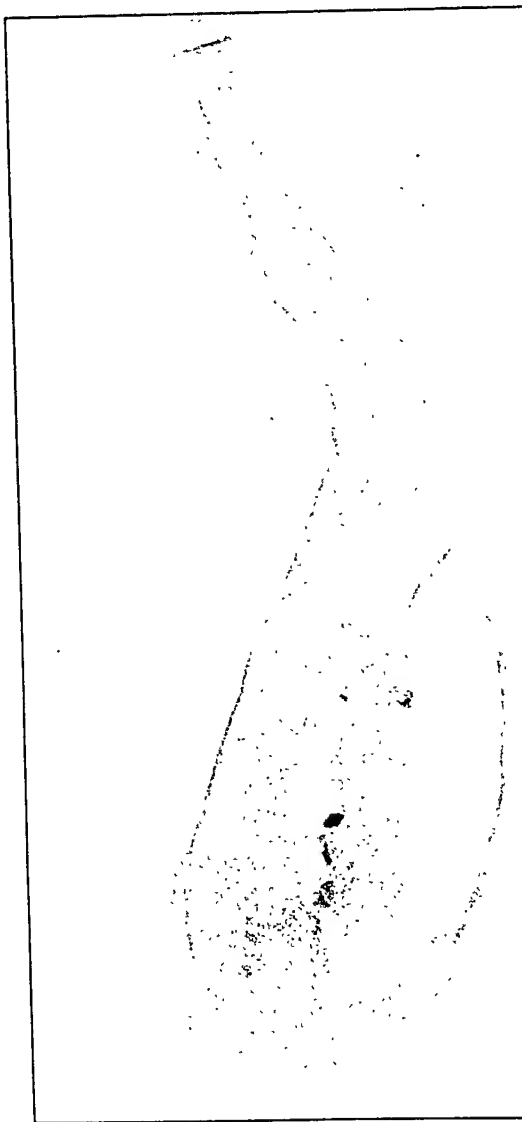


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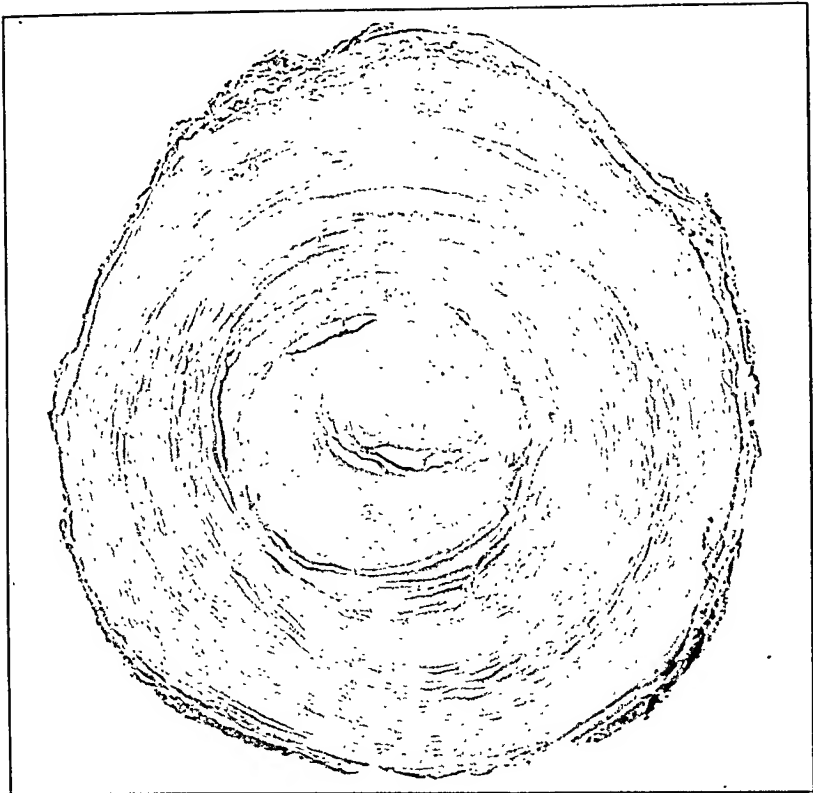
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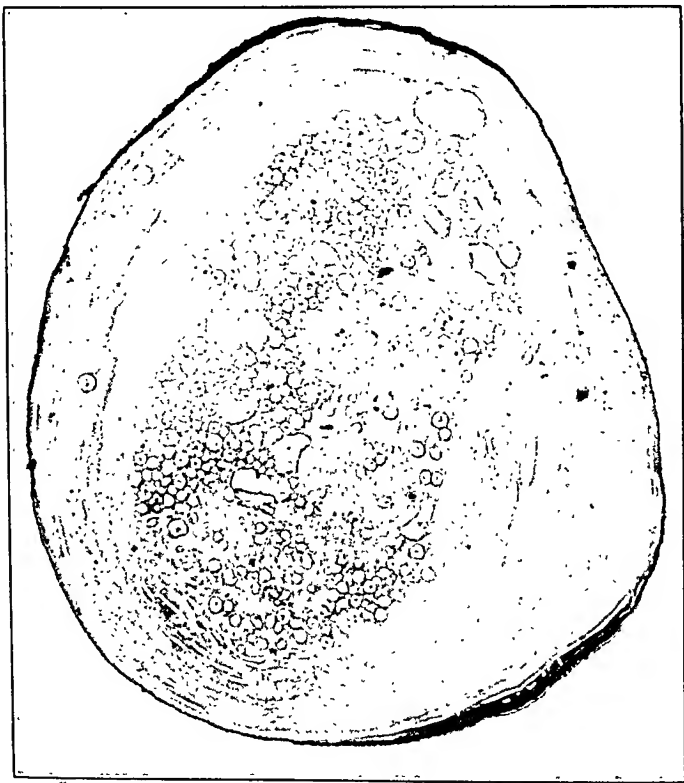
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PLATE 100

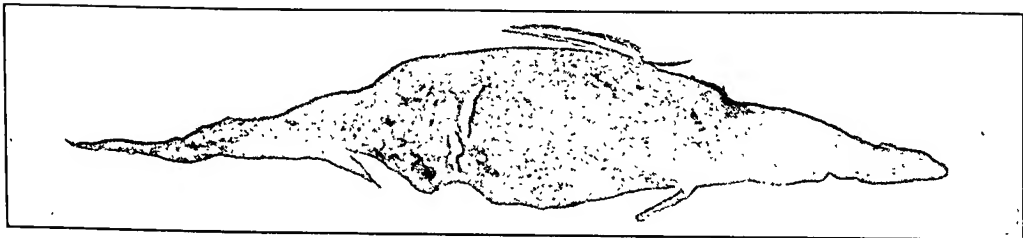
FIGS. 5 to 7, inc. Calcified, free, fibrous bodies in microscopic section. Figs.
5 and 6 $\times 40$. Fig. 7 $\times 35$.



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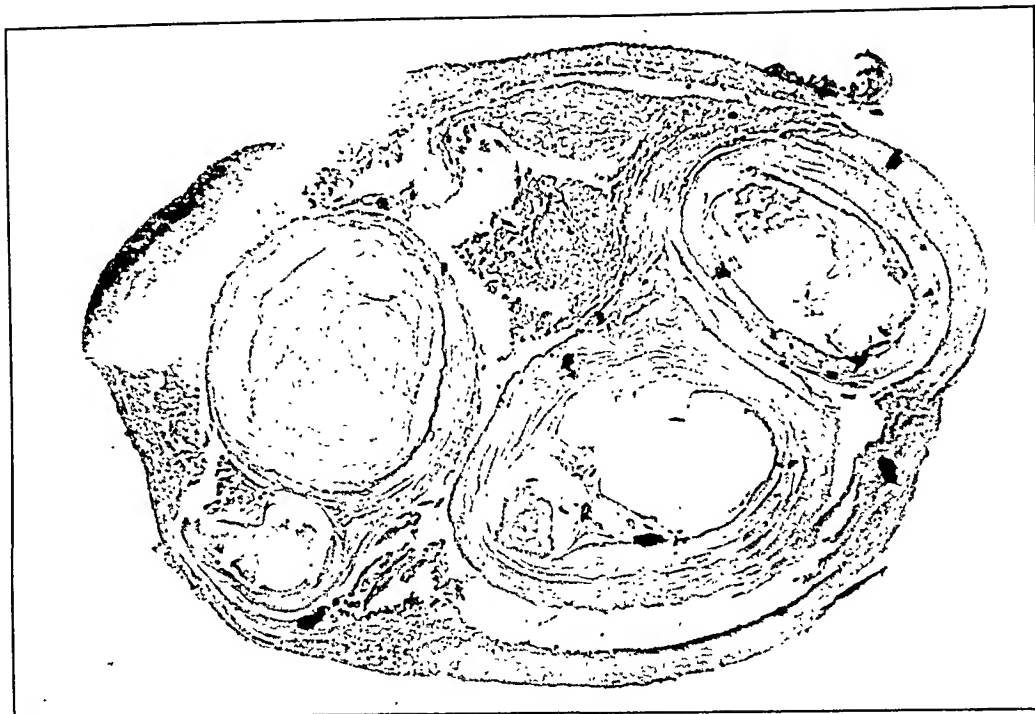
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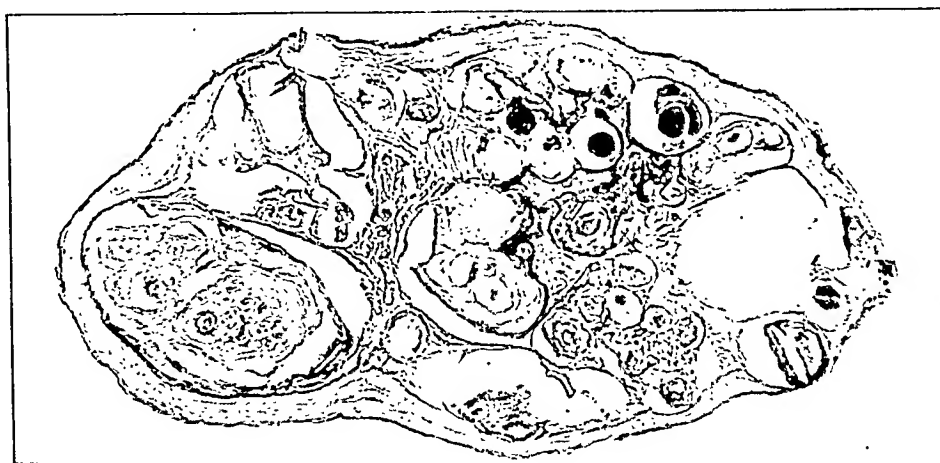
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PLATE 101

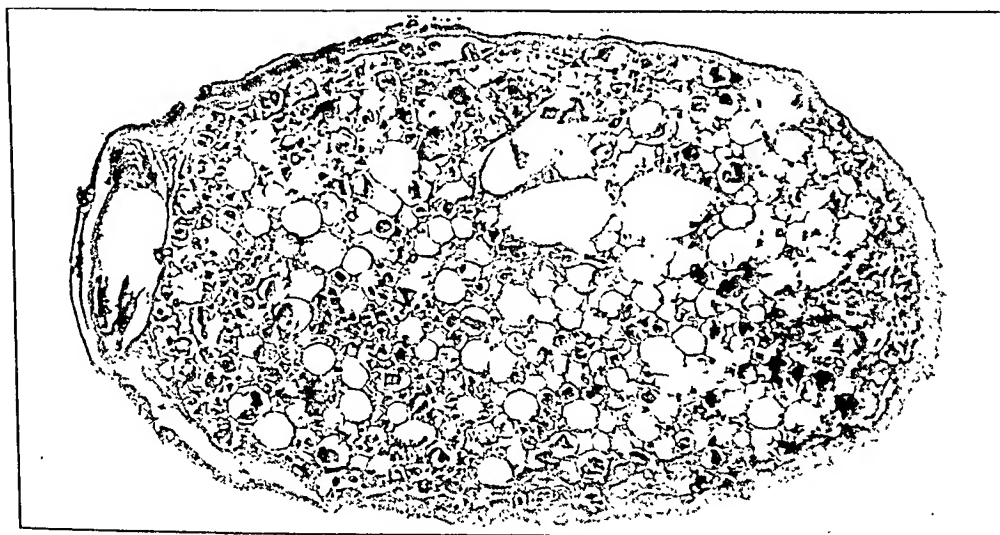
FIGS. 8 to 10, inc. Multilocular, partly calcified, free bodies, each containing numerous calculi, the larger of which seem to be fusion products. Fig. 8 \times 50. Fig. 9 \times 70. Fig. 10 \times 40.



8



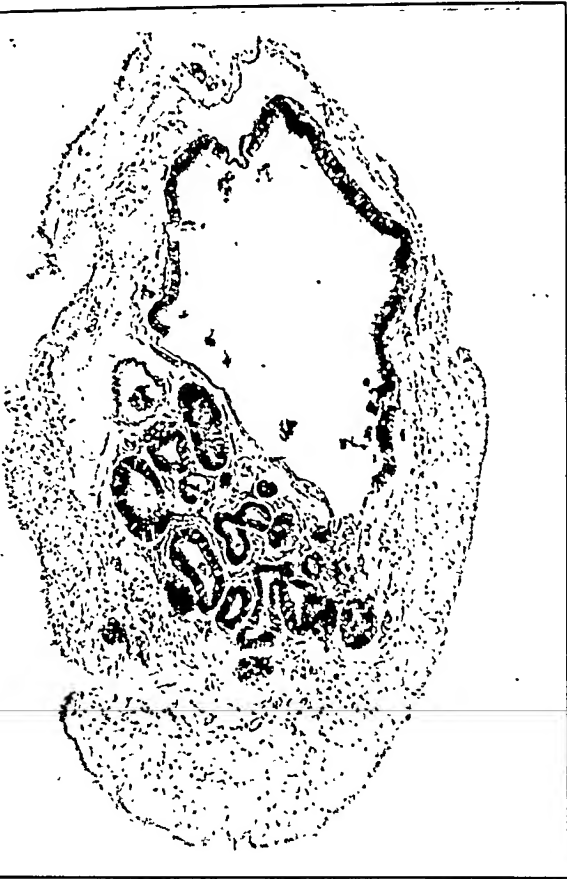
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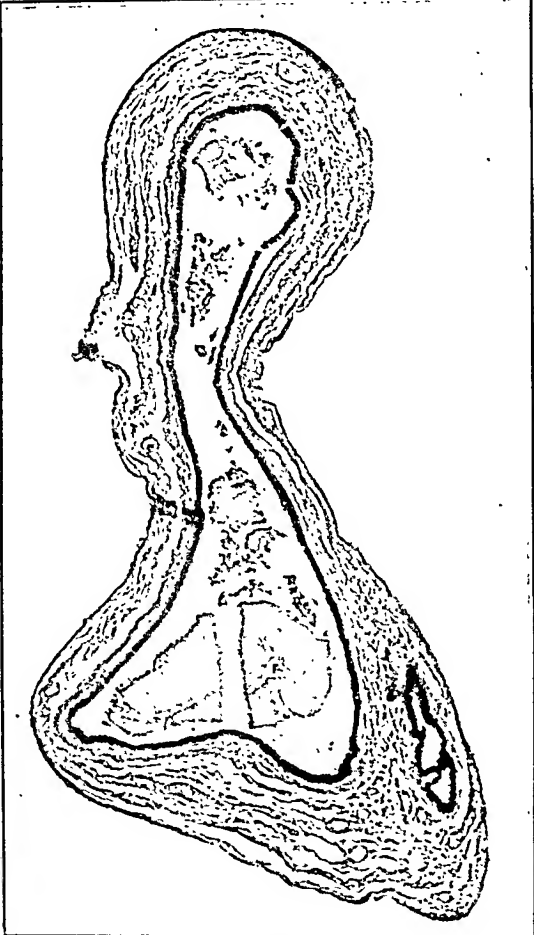
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PLATE 102

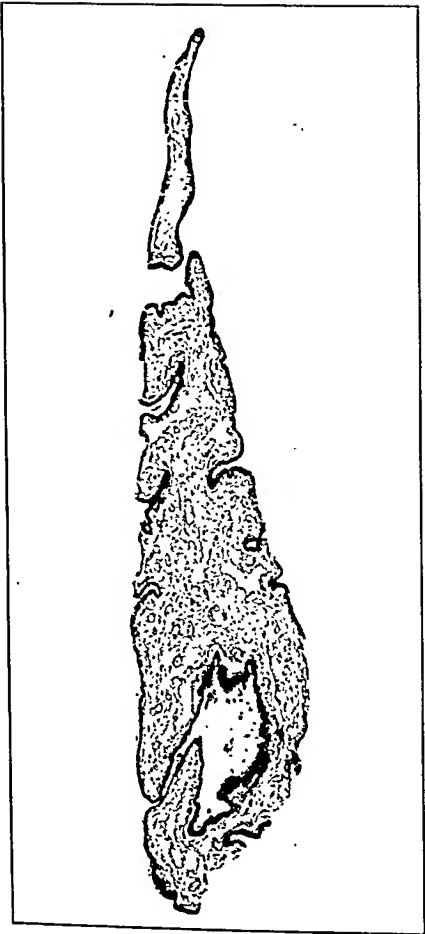
- FIG. 11. A cross-section of an appendix testis with numerous tubules. $\times 60$.
- FIG. 12. A cross-section of an appendix testis as usually described, representing the hydatid type. $\times 50$.
- FIG. 13. A cross-section of a plicated, fibrous form of appendix still covered with mesothelium. The latter is high cubicle and even columnar in type in the invaginations. $\times 35$.
- FIG. 14. A cross-section of a fibrous appendix testis covered by high cubicle mesothelium. $\times 90$.



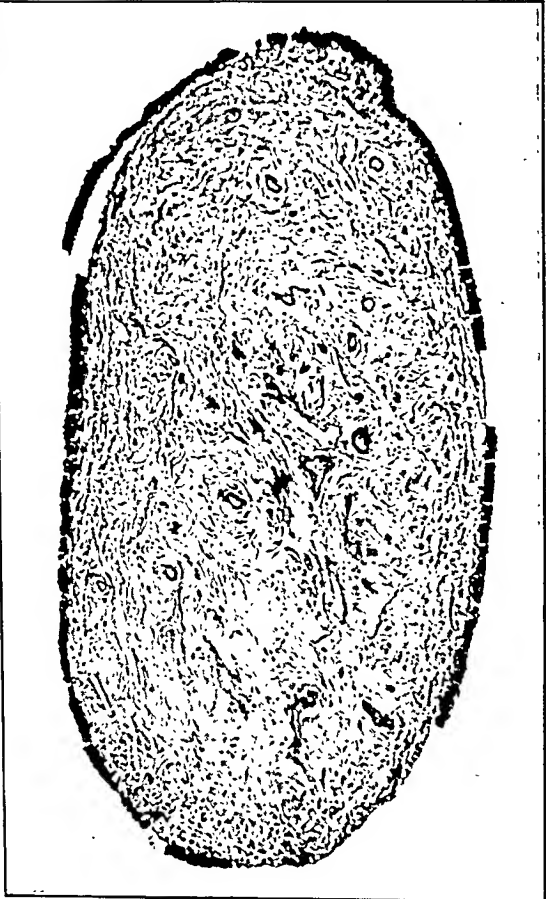
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14

Meyer

Corpora Libera in Tunica Vaginalis Testis

CALCIFICATION OF THE SUPRARENAL GLAND *

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Calcification and ossification are extremely rare in the human suprarenal gland. Brüsche¹ noted calcification in the suprarenals of fifteen out of 303 cats. Marine² reported sixty-four instances in 257 felines and suggested that the toxin of distemper caused focal necrosis of the cortex with subsequent calcification. The process in both instances was bilateral and no ossification occurred. Kruse³ noted bone formation in the medulla of the suprarenal gland of a monkey. In humans, calcification has been noted in tuberculosis of this gland (MacCallum,⁴ Kovács⁵). Wooley⁶ described a case of bone and bone marrow formation in a case of tuberculosis of the suprarenal. Tubercles were present around and in the areas of bone formation. Hemorrhage with subsequent calcification has also been noted (Adami and Nicolls,⁷ Surbek,⁸ Victor⁹). In a boy 14 years of age, who died of malignant lymphogranulomatosis, Harbitz¹⁰ reported calcification of the liver, lungs, kidneys, suprarenals, lymph nodes and capsule of the thymus. Newsam¹¹ noted ossification of the entire medulla of both suprarenals with marked thinning of the cortex in a girl of 2 years and 7 months who suddenly developed signs of suprarenal insufficiency with fatal termination. Harbitz mentions a case of a deposit of calcareous material with degeneration of suprarenal parenchyma in a case of parathyroid hyperplasia.

Marine found no evidence of calcification in the reticular layer of the cortex in the pig, ox, sheep, dog, rat or rabbit corresponding to the type he found in cats. He thought that the toxin of distemper was the specific cause. Freezing with ethyl chloride and injury to the gland with diphtheria toxin or arsenic gave negative results in the rabbit and cat. Experimentally, calcification has been produced in the kidney tubules, stomach, lungs, and myocardium by intravenous injection of calcium (Tanaka¹² and Katase¹³), overdoses of parathyroid hormone (Hueper¹⁴) and by acid diets (Dreyfuss¹⁵). The suprarenal gland has never been the seat of calcification in any

* Received for publication June 4, 1928.

of these experiments. In "metastatic calcification" also, the suprarenal has not been involved in any of the reported cases.

In this report we have collected the human cases having a distribution of calcium deposits in the suprarenals similar to that found in cats by Marine and Brüscheweiler. All the material was obtained from the autopsies at Montefiore Hospital. The patients were practically all adults who died from chronic diseases. In none of the forty-six cases of tuberculosis of the suprarenals in our series was calcification found. It occurred, however, in a number of instances in the blood vessels and also in areas of infarction in several suprarenal glands. The routine examination of the suprarenal glands in 1185 autopsies revealed four instances of calcification of the reticular layer similar to the lesions described in cats by Marine. These are here reported. The process was diffuse in three instances and in the other only a spicule of calcium was found. Very little connective tissue or other signs of chronic inflammation were found. Sections of the other gland (in two instances) revealed no other deposits of calcium salts. A soft deeply staining calcium deposit was interpreted as of recent origin and hard pale staining, lighter blue areas were assumed to be older deposits.

CASE 1. Clinical History: M. K. Autopsy No. 3419, male, 60, Russian. No history of infectious disease, and apparently was well until three years before his death when all his teeth were extracted on account of ulcerating tissue of the mouth. This ulceration proved to be due to a tumor. He received X-ray and radium therapy. Course of his disease was afebrile.

Anatomical Findings: Carcinoma of the soft palate with metastases to the bones of the skull, calcified tuberculosis of the bronchial lymph nodes, bronchopneumonia. The kidneys showed large calcareous deposits in the collecting tubules. Calcification was present in the intima of the aorta.

Suprarenal: Large area of calcium deposition in the reticular and fascicular layers of the cortex arranged in dense clusters of fine dots and strands around the cells. The calcium in some areas was deep staining. No inflammatory exudate or increase in connective tissue noted.

CASE 2. Clinical History: J. L. Autopsy No. 3837, male, 49, born in United States. Mother died at 29 of a "heavy cold." One brother died at 6 months. One sister died at 1½ years. Married at 41. Several children alive and well. Pertussis, malaria at 16, syphilis at 26. He constantly held his pipe in the lower left angle of his jaw. All his teeth were extracted during the course of his illness. Patient ran a slight fever (never over 101°F) for several months. Wassermann + + + +, blood sugar 127 mg., urea 11.2 mg., uric acid 3.7 mg.

Anatomical Findings: Epithelioma of cheek, with involvement of the maxilla, luetic aortitis, bronchopneumonia.

Suprarenal: Microscopic area of calcium deposit in the reticular layer of the cortex, arranged in form of dense strands, light stain. No evidence of inflammatory reaction.

CASE 3. Clinical History: F. F. Autopsy No. 4195, male, 44, Russian. Previous history not obtained in detail. For one and a half years he complained of diarrhoea, weakness and loss of weight. Physical examination revealed a cachectic individual who had a colostomy. Course was afebrile.

Anatomical Findings: Carcinoma of the rectum with metastases to the liver, spleen, regional lymph nodes with extension to colon, ileum and bladder, bronchopneumonia. Microscopically one kidney showed large calcareous deposits in the collecting tubules.

Suprarenals: Marked deposition of calcium in the reticular layer of one suprarenal. No calcification found in the other. Very dense calcium deposits with a thin connective tissue capsule and trabeculae extending into the calcified areas.

CASE 4. Clinical History: J. S. Autopsy No. 4247, female, 41, Russian. Mother died of cancer of the stomach. Measles, mumps, pertussis in childhood. Pleurisy and pneumonia two years before. Patient had cough with blood-streaked sputum at 18 years. Enlarged glands with pruritus for seven years. She developed a right otitis media and terminal sepsis. Blood sugar 100 mg., hemoglobin 68 per cent., blood urea 17 mg. She received very little X-ray therapy on account of low white blood count. She ran a terminal septic fever for six weeks.

Anatomical Findings: Splenomegaly of undetermined origin, extensive bronchopneumonia in right lung, healed tuberculosis of right upper lobe. Microscopically the spleen showed marked proliferation of the reticular and endothelial elements.

Suprarenal: Marked deposition of calcium in the reticular layer of the cortex, similar to Case 3 excepting that calcium deposition was not so marked and there was less connective tissue. Calcium was light staining and present in only one gland.

DISCUSSION

The etiological factor in the formation of this particular lesion has not been satisfactorily established.

It is easy to assume that hemorrhage in the suprarenal with subsequent calcification would account for it. Calcification in areas of hemorrhage has occurred in several reported instances, but calcification is very rare considering the frequency of suprarenal hemorrhage in the newborn. It is possible that an extensive but sublethal hemorrhage, at birth, might go on to calcification in the process of healing. Such deposits might then remain throughout life as in cats. In favor of this view is the localization of the calcification in the reticular layer of the cortex. Against this is the absence of calcification in the medulla.

Calcification as a terminal process of resolution in areas of necrosis such as occurs in tuberculosis, syphilis, and other chronic inflammatory lesions is of common occurrence. Such a lesion does not appear to have been a factor in the cases here reported. The absence of marked connective tissue proliferation and other signs of chronic inflammation would tend to substantiate such an opinion.

An embryonal metaplasia may account for the presence of bone in the cases of Newsam and Kruse. It would be difficult to explain the observation of Wooley in this way, although the tuberculous process might have secondarily involved the suprarenal gland.

Disturbances in the acid-base ratio may lead to calcification in other organs, but it has not yet been produced in the suprarenals in this manner.

A disturbance of function of the glands of internal secretion, particularly the parathyroids and thymus, could be an etiologic factor as suggested by Harbitz.

Degenerative changes in the renal epithelium with deposits of lime salts are frequently seen in localized X-ray radiation of these organs (Warthin,¹⁶ Hartman, *et al.*¹⁷). It is possible that a similar result might occur in the suprarenals. However, in our series, the suprarenal area was irradiated in only one of the cases with one half the erythema dosage. I do not believe that X-ray radiation played any rôle in the calcification of the suprarenals in these cases.

The presence of a specific toxin of bacterial or other origin could cause necrosis with subsequent calcification of the suprarenal. Since distemper is the most important etiologic factor in the calcification of the cat's suprarenals it is possible that the somewhat comparable disease (influenza) in man which is known to markedly affect the suprarenals may have been a factor in our human cases. In our series it was not possible to obtain any evidence implicating influenza.

SUMMARY

Four cases of calcification of the reticular layer of the cortex of the suprarenal gland in 1185 autopsies in a hospital for chronic diseases are here reported. Several possible etiologic factors are discussed.

I am indebted to Dr. David Marine for his many helpful suggestions.

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DESCRIPTION OF PLATE

PLATE 103

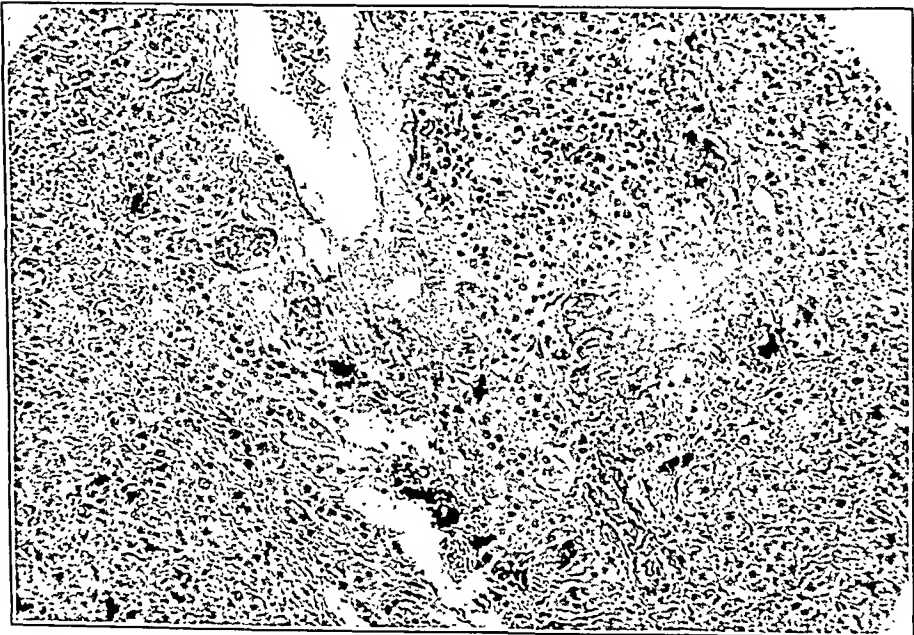
- FIG. 1. Case 1, photomicrograph of a recent area of calcification in the reticular layer of the cortex. $\times 40$.
- FIG. 2. Case 3, photomicrograph showing extensive areas of calcification in the reticular layer of the cortex. $\times 40$.
- FIG. 3. Case 3, photomicrograph of a small area of Fig. 2 showing crystalline deposits of calcium salts in fascicular and reticular layers of cortex. $\times 200$



1



2



3

STUDIES ON THE BONES IN AVIAN RICKETS *

I. BONE LESIONS IN CHICKENS DEPRIVED OF THE ANTIRACHITIC FACTOR AFTER FIVE WEEKS OF NORMAL GROWTH

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When chicks receiving a ration poor in antirachitic vitamine are deprived of direct sunlight they develop after a few weeks a condition known as "leg weakness." As implied by the name the disease is characterized by marked difficulty in standing and moving, usually accompanied by growth retardation and symptoms of anemia. Morphological changes, some of which are only apparent at late stages of the disease, have been described by several investigators. Disturbances in the processes of ossification manifested in production of abundant osteoid, spontaneous fractures and beading of the ribs have been described by Doyle.¹ The parathyroids appear enlarged (Doyle²), and in advanced cases of the disease their parenchyma shows regressive changes (Nonidez and Goodale³). Finally, there are also metabolic changes, the most important of which are low phosphorus and calcium content in the blood (Hart *et al.*;⁴ Ackerson *et al.*;⁵ Steenbock *et al.*;⁶ Hughes *et al.*⁷) and in the bones (Hart *et al.*⁸). It is not surprising then that leg weakness has been generally regarded as the equivalent of mammalian rickets.

As a result of comparative studies of bone lesions in leg weakness and in experimentally produced low phosphorus rickets in rats, Pappenheimer and Dunn⁹ concluded that leg weakness is not rickets. "An excess of osteoid tissue, perhaps the most significant and distinctive feature of rachitic bone, was never present in the chick preparations. Indeed, the amount of osteoid seemed diminished in comparison with that normally found in the healthy growing control of the same age." (Cf. p. 722.) The age of the chicks examined ranged from 16 to 37 days. They were placed under experimental conditions soon after hatching.

Hughes and Titus¹⁰ have pointed out that Pappenheimer and Dunn failed to find typical rachitic changes in the skeleton because these investigators examined only very young chicks. They have

* Received for publication May 18, 1928.

also suggested that the differences between the microscopic bone lesions of leg weakness and experimental low phosphorus rickets in rats may be due to the fact that the same disease produces different histological changes in species as widely diverse as the chicken and the rat.

The present study of the bones of chickens affected with leg weakness at different ages furnishes an explanation of the divergence of opinion that exists on the subject. But, in order to understand properly the effect of lack of the antirachitic factor on the skeleton, its condition at the time of hatching and during the first weeks of life must first be reviewed.

In the newly hatched chick, as shown by several of the early authors and more recently by Fell ¹¹ ossification is little advanced and there is an abundance of embryonic cartilage at the ends of the diaphyses of the long bones. The latter consist of cylinders of firm periosteal bone enclosing the marrow and unreplaced cartilage. At this stage the spongiosa is represented by a few bony trabeculae of endochondral origin placed under the periosteal bone at the diaphyseal ends. The bulk of the spongiosa develops, therefore, after hatching. In the long bones of adult chickens the spongiosa is well developed in the diaphyseal ends but its trabeculae are thinner than the corresponding structures of mammalian bones.

The development of the spongiosa is preceded by the penetration of numerous vessels from the marrow cavity into the almost continuous band of embryonic cartilage. The invading vessels cut the band into a series of irregular strips, more or less parallel to the axis of the bone; the bases of the strips are continuous with the zone of proliferation. The strips just mentioned form the zone of provisional calcification. Bone is deposited around each strip and at first appears as an osteoid margin. This in White Leghorns at least does not reach the bases of the strips until the end of the fourth or the middle of the fifth week after hatching. At this moment most of the cartilage present in the diaphyses is of recent formation in the zone of proliferation, the embryonic cartilage having been replaced by bone, and the bones have only now reached a condition corresponding to that in newly born mammals.

From these considerations it is plain that although to external appearances newly hatched chicks are in a more advanced stage of development than most of the newly born mammals used for labora-

tory work — if we judge from their general activity and the development of their sense organs — their bones are in a condition only paralleled in the mammal during fetal life. *A priori* it is questionable whether comparison of the lesions present in juvenile avian leg weakness with the lesions in experimentally produced mammalian rickets is fair since the treatment begins in each case when the bones are in different stages of development. There are, moreover, other factors that have an important bearing on the skeletal changes observed in early stages of the disorder produced by lack of the anti-rachitic factor on chickens.

Under the adverse conditions of continuous existence indoors and a deficient diet, growth of the bones and replacement of their cartilage by endochondral bone are retarded in young chicks. The extent of this retardation varied in our experiments according to the diet. Chicks that received a basic ration* not supplemented with semisolid buttermilk were very small at six weeks and few survived after this period. The condition of their bones was very similar to that described by Pappenheimer and Dunn and can be scarcely spoken of as "rachitic." Although the band of embryonic cartilage has been cut into strips by the vessels from the marrow cavity, as happens in normal chicks, the strips lack a well developed osteoid margin. The spongiosa is represented by a few trabeculae; hence the osteoporotic aspect of the bones. When, on the other hand, semisolid buttermilk was added to the diet better growth was obtained but rachitic lesions were not present during the first six weeks of life, although the processes of ossification were more normal than in the chicks not receiving buttermilk. Lesions appeared, however, at a later age, and were very pronounced in two birds kept continuously indoors for four and a half months. Apparently, growth retardation though not so marked as in the case mentioned above prevents the early appearance of such rachitic changes as enlargement of the ends of the long bones, beading of the ribs, and formation of abundant osteoid since the development of these abnormalities depends on active growth.

With these facts in mind it became apparent during the course of the work that the problem of the relation of leg weakness to mam-

* The basic ration used in the experiments was scratch feed containing equal parts of yellow corn, wheat and oats, and a dry mash made up of yellow corn meal, bran, sifted ground oats, white middlings and meat scrap with about 12.5 per cent of bone.

malian rickets could not be satisfactorily solved when newly hatched chicks are used for the experiments. Obviously, from the comparative standpoint, the ideal requirements for a study of the problem are realized when the treatment is applied to chickens previously allowed to grow normally for a period of five weeks. At this time, as already stated, the bones are in a stage of development that closely resembles the condition of mammalian bones during the first weeks of life, and any disturbing influence of the early stages of growth can be easily eliminated.

In the present paper the results of the experiment outlined above will be reported. Thirty-three White Leghorn chicks, hatched May 31, 1926, received exposures to sunlight, when available, during the first five weeks of life. The length of exposure was not uniform but averaged about fifteen minutes daily in the first week; this was increased to about one hour when the chicks were three weeks old. A diet consisting of scratch feed and dry mash was fed.* In order to obtain better growth the diet was supplemented with semisolid buttermilk for four weeks. During the fifth week this was gradually discontinued. At the end of the fifth week four chickens were autopsied and some of their bones preserved for histological study. The other birds were confined in a brooder house, where they could not receive direct sunlight. They were fed the same diet as before, with the exception of the buttermilk, which had been previously withdrawn from the ration.

Twenty-three chickens were autopsied after four to five weeks of existence indoors, while six birds, exhibiting diverse degrees of leg weakness, were placed in a chicken house opening into a yard, where they could receive sunlight at will. The condition of these birds soon improved; two of them were killed after two weeks, the others after six weeks of existence in a normal environment. Their bones were preserved for study.

The writer wishes to acknowledge the spirit of coöperation shown by Mount Hope Farm, Williamstown, Massachusetts, where the experiment was conducted under the direction of Dr. H. D. Goodale, to whom he is also indebted for suggestions and criticism.

* The same basic ration mentioned in previous footnote was used in this experiment.

I. GROSS SKELETAL CHANGES

While the combined effect of lack of sunlight and a diet deficient in antirachitic vitamine during the first weeks of life is manifested in decreased growth rate, the growth of chickens receiving the treatment at a later age was little affected and their size was normal for their age. Symptoms of leg weakness began to appear as early as the third week after exposure to unfiltered sunlight was discontinued. In this respect there seems to be little difference between very young chicks and older birds. Various degrees of leg weakness occurred, from an almost complete inability to move about to a nearly normal condition in which the symptoms of the disease were visible only when the chickens tried to jump, or while they were scratching in search of food.

There was, however, a marked contrast when the bones of the chickens in the present experiment were compared with those of chickens deprived of the antirachitic factor shortly after hatching. As already stated the bones of the latter do not show gross rachitic lesions after six or even eight weeks of lack of the antirachitic factor, whereas in the birds of the present study there were deformities similar to those described by Doyle in pullets and laying hens. The ends of the tibiotarsus and femur were slightly enlarged at the close of our experiment in almost all of the twenty-three birds examined. In two males and one female the distal ends of the sternal and vertebral ribs * showed knobs on their inner surface, comparable with the characteristic "beading" of the ribs in the rachitic mammal. Another feature, reported by Doyle, was present in three males and two females; this abnormality consisted in a bending in of the articulation of the two portions of the ribs, which produces a groove in the corresponding thoracic side. In our birds, however, this defect was not bilateral, but was restricted to the left side of the body and in some cases was associated with beading of the ribs. Finally, deformity of the keel of the sternum occurred in five males and one female; all the birds with beaded ribs showed this abnormality. In a general way, judging from the data at hand, it seems that rachitic changes appear earlier in the males; this may be due to the fact that

* The ribs of birds consist of two independent portions held together by fibrous tissue, one articulated with the vertebrae (vertebral ribs) and the other articulated with the sternum (sternal ribs).

the latter grow faster and attain heavier weight when adult than the females.

Spontaneous fracture of the ribs, observed by Doyle in mature chickens, was not present in any of the birds of the experiment described in this paper.

A noteworthy feature of leg weakness in the domestic fowl is the absence of bending of the bones of the legs. At least, bent bones were observed in only one series of the several experiments conducted at Mount Hope Farm and then were not restricted to the chickens deprived of the antirachitic factor.*

Gross frontal sections of the tibiotarsus and femur showed the presence of a transverse band of pink color, situated under the zone of proliferation. This band (Fig. 1, *r*) represents the bone formed during the experimental period and owes its light color to the absence of normal marrow. Its depth varied a good deal in the seventeen chickens examined, but it was never absent. The bone was so soft at this level that it could be cut with the scalpel. Bones of normal chickens lack the band just described since the marrow is well developed throughout.

II. MICROSCOPIC CHANGES

The femur, tibiotarsus and, in a few cases, the articulation of the sternal and vertebral ribs, were preserved and decalcified in picronic acid for twenty-four to thirty hours. They were washed in running water and embedded in parlodion. Sections were stained with Mann's acid hematein (with or without counterstain) and Rio Hortega's silver carbonate method. The latter gave good results for the study of the finer structure of the osteoid and the reticulum of the marrow, especially when the sections were toned with gold chloride. But, perhaps due to the method of preservation, the cellular contents of the marrow, cartilage and bony trabeculae were not impregnated. When desired, these cells were brought out through counterstaining with indigo carmine.

* In the first experiment bent leg bones occurred in chickens deprived of the antirachitic factor and also in chickens exposed to direct sunlight, irradiated with a mercury-vapor lamp or fed the basic ration supplemented with cod liver oil. As suggested by Goodale¹² the condition of their bones may have nothing to do with lack of the antirachitic factor.

Examined under the microscope, the transverse band of bone formed during the period of existence indoors is seen to consist of broad osteoid trabeculae. The trabeculae are richly anastomosed and may show remnants of unreplaced cartilage. The excess of osteoid in the rachitic band can be well appreciated through comparison of similar regions of the same bone in normal and rachitic chickens. Since the chickens examined were deprived of the anti-rachitic factor for only four to five weeks, enlargement of the ends of the long bones was not very marked. The depth of the zone of columns of flattened cells and of the zone of hypertrophied cartilage cells (zone of provisional calcification) is very unequal in some specimens suggesting that the process of cartilage replacement by bone was not proceeding at a normal rate. In beaded ribs the bony part is composed of unusually thick trabeculae of osteoid, surrounded by a well defined and, in some places, thickened osteoblastic layer. The zones of proliferation and provisional calcification appear distorted and, in one particular case, replacement of cartilage by bone has taken place in such an irregular fashion that the original band of cartilage has been disrupted and now consists of a distal portion and two lateral masses of unreplaced cartilage, situated more posteriorly (Fig. 2, c' , c''). Growth of this displaced cartilage apparently causes the beading so characteristic of rachitic ribs.

The epiphyseal cartilage in these rachitic bones is moderately calcified. The areas of calcification occur in the center of the cartilage, except in the immediate vicinity of the epiphyseal vessels. The presence of calcium in the cartilage under consideration, emphasized by Pappenheimer and Dunn in young chicks, can be accounted for when we keep in mind that since in the fowl there are not independent epiphyseal ossifications,* the necessary mechanism for erosion and removal of preëxisting cartilage seems to be absent during the phase of active growth. Ossification of the epiphyseal ends in the long bones of birds takes place when the bones cease growing. Inactivity in the zone of proliferation soon results in its invasion by vessels and osteoblasts, which enter the epiphyseal cartilage from the diaphysis and destroy the calcified matrix, after

* In the epiphyseal cartilage covering the lower end of the tibia there are, however, two independent centers of ossification. These centers are not true epiphyses but represent tarsal elements fused with the tibia; hence the name "tibiotarsus" applied to the latter in most of the treatises on comparative anatomy.

which bone is deposited. It seems likely, then, that the cartilage under discussion became calcified during the first weeks of life and that it remained as such.

Periosteal ossification is very active at the level of the diaphyseal ends. The osteoblastic layer of the periosteum is very thick in this region (Fig. 3, *ol*) as compared with the corresponding layer in normal bones. Evidences of increased bone formation at this level are manifested in rachitic bones in the presence of thick trabeculae more or less at right angles to the surface of the bone. These trabeculae (Fig. 3, *o*) show different shades of pink in slides stained with hematein and eosin. Toward the center of the shaft they are deeply stained and possess small, irregularly shaped osteocytes, while toward the periphery of the bone the trabeculae are much paler and show larger cells; in this region they gradually fade into the osteoblastic layer of the periosteum. At the diaphyseal ends, periosteal bone, in both normal and rachitic chickens, contains stout collagen fibers from the capsule and ligaments of the joint. These fibers are best seen in slides stained with silver carbonate.

Since the use of silver carbonate has revealed interesting features in both the osteoid and the marrow it seemed convenient to describe them in detail. Whether some of the abnormalities observed are restricted to the fowl or whether they also occur in mammalian rickets I am unable to say, inasmuch as the method is comparatively new and, as far as I am aware, has not been used in the histopathology of rickets.

Formation and Characteristics of the Osteoid in the Rachitic Band: Rio Hortega's silver impregnation method stains collagen fibers in the bone matrix (decussating fibers of Sharpey of the mammalian anatomy) and, at the same time, impregnates the fibers of the externa of the vessels and the reticulum of the marrow. The latter appears stained deep purple or black in sections toned with gold chloride while collagen fibers stain light purple.

Some of the collagen fibers of the externa of the vessels are deflected and run freely in the marrow spaces where they act as the main support for the delicate network of the reticulum. In some areas they seem to be continuous with the osteoid fibers.

In common with the bones of rachitic mammals the bones in avian rickets show a thick osteoblastic layer, several cells deep in many places. The osteoblasts are closely packed and the continuity

of the layer is interrupted only by the presence of multinucleated giant cells (osteoclasts). For the most part the osteoblasts have round or oval eccentric nuclei, and their size is variable, from a fairly small cell to abnormally large elements. These overgrown or hypertrophied cells possess relatively small, deeply staining nuclei and are incorporated into the bone matrix (Figs. 8 and 10, *o'*). Large osteoblasts were not found in the bones of those chickens autopsied before the experiment was begun. Furthermore, in rachitic birds they are absent in those portions of bone which were laid down prior to the experiment. The presence of hypertrophied osteoblasts seems, therefore, closely associated with the formation of abundant soft osteoid, and to be one more item due to lack of the antirachitic factor.

The structure of the matrix differs somewhat in bone trabeculae deposited before and during the experiment. In the former the fibers are thin and parallel; the outlines of individual fibers are often difficult to see since they are cemented together by an interfibrillar substance in which calcium salts are presumably deposited. In rachitic areas the fibers are of irregular thickness and in some places they exhibit a tendency to form definite bundles. Furthermore, in many areas they are not parallel but run in different directions. The histological picture suggests a disorderly arrangement of the fibrous material (Fig. 4, *o*).

In normal bone recently formed osteoid around the prolongations of cartilage emanating from the zone of proliferation appears under the aspect of a well defined osteoid margin (Fig. 7, *om*). The osteoid margin is also present in rachitic bones, but it is usually somewhat thicker (Fig. 8, *om*). In normal bone the osteoid margin gradually increases in thickness, but the osteoblasts never penetrate the calcified cartilage of the prolongations that constitute the zone of preliminary calcification. In sections stained with silver carbonate the fibers run parallel and they are closely applied against the surface of the cartilage (Fig. 5). Here and there, however, one or several lacunae of the cartilage have been partly corroded and osteoblasts are seen in their interior, together with much bent fibers which fill the lacunae and give the impression of a complicated intralacunar network.

In rachitic bone, on the other hand, the osteoblasts freely enter the cartilage of the prolongations mentioned above and form fibers

running in every direction. In this way a very coarse network of fibers enclosing cartilage cells is produced (Fig. 6). The penetration of osteoblasts and the formation of intracartilaginous fibers are probably favored by the absence of calcification of the cartilage.

In certain areas within the osteoid trabeculae there are groups of hypertrophied osteoblasts. Large multinucleated cells closely resembling the osteoclasts also occur within the trabeculae. It was thought at first that such cells might be true osteoclasts which had penetrated the trabeculae and that the absence of any erosion suggesting the path of their entrance into the osteoid could be explained on account of the plane of the section. Repeated observations, however, indicated that in no case was there communication with the intertrabecular spaces. Finally, it was clear that intra-osteal multinucleated cells arise *in situ* as a result of migration and coalescence of hypertrophied osteoblasts. An early stage of their formation has been represented in Fig. 9. In all probability lack of calcified cementing substance between the osteoid fibers enables the osteoblasts to glide along the interfibrillar spaces and gather in groups. It cannot be denied, however, that the multinucleated cells may arise as the result of a combined process of division and coalescence of free osteoblasts, but mitotic or amitotic processes, suggesting that cell division plays an important part, were never found in the slides. Absence of corroded areas of osteoid around the large multinucleated cells strongly suggests that these elements are not active bone destroyers, and in spite of their similarity to the osteoclasts they are probably a different type of cell arising as a result of the osteoblastic hyperplasia prevalent in the rachitic band.

Changes in the Marrow: Lack of the antirachitic factor prevents the development of normal marrow during the experimental period but does not cause degenerative changes in the marrow already present in the bones at the beginning of the experiment. On the contrary, instead of degeneration there is a tendency toward hyperplasia of the fully differentiated marrow. Hyperplastic processes are not general, but restricted to small foci that appear as nodules made up of much crowded cells. In slides stained with hematein-eosin the nodules somewhat resemble lymph nodes, known to occur in the marrow in some cases of human rickets (Schridde¹³). Close study, however, shows that lymphocytes are absent and that many cells contain eosinophilic granulations. Undoubtedly, the

nodules under discussion are the source of cells of the leucoblastic and erythroblastic lines. They occur chiefly in the vicinity of the rachitic band. It does not seem unlikely that hyperplasia in this case is of a compensatory character, inasmuch as blood-forming elements are very scarce in the marrow of the rachitic band.

The aspect of the marrow in the rachitic band is very similar to that of the corresponding structure in rachitic mammalian bones. The spaces between bony trabeculae are occupied by large numbers of loosely arranged, spindle-shaped cells, oriented in every direction save in the proximity of the trabeculae where the predominant direction is parallel to the trabecular surface (Fig. 10). A certain amount of fibrous tissue also exists around those trabeculae of the spongiosa deposited prior to the experiment, and also on the inner surface of the cortex. The atypical marrow in these locations has apparently taken the place of bone reabsorbed during the process of enlargement of the central cavity of the bone. Since the bony trabeculae in chickens are more slender and not so numerous as in mammalian bones the intertrabecular spaces appear larger than the corresponding spaces of the mammal, even though there has been marked thickening of the trabeculae formed during the experimental period.

The marrow within the rachitic band does not contain fat cells, and myelogenous elements form discrete groups in which a few myeloblasts and myelocytes are recognizable. The spaces between the spindle-shaped cells seem to be filled with a watery fluid. Lack of myelogenous tissue causes the pink color of the rachitic band in sections of fresh bones, as compared with the red color in normal bones.

Structural differences between normal and rachitic marrow are further illustrated by slides stained with silver carbonate and toned with gold chloride. Figs. 11 and 12 represent normal and rachitic marrow, respectively, from the same section. For the sake of clearness the faint cell outlines have not been indicated. The normal marrow at the right of the capillary (*bv*), in Fig. 11, contains fat cells; at the left it is much more compact. This compact area is a hyperplastic nodule. It will be noticed that the reticulum in the latter is very well developed, while in the region with fat cells reticular fibers are not so abundant. Collagen fibers (*co*), arising from the externa of the vessels, form the main support of the mesh-work.

The outstanding fact in the marrow within the rachitic band is the condition of the reticulum (Fig. 12, *r*). In order to appreciate fully this difference it must be stated that both figures in the Plate are camera lucida drawings made under the same magnification. Photographs were not made since the meshes of the reticulum would not be clearly seen in a single plane. Fine fibrils, such as are seen in normal marrow, are absent or poorly developed. Instead, there is a meshwork of faintly stained, coarse trabeculae. In hematein-eosin slides the reticulum is not clearly visible but its thicker portions are seen as indistinct pink masses suggesting colloid. At first it was thought that these masses were due to degenerative changes in the marrow, but the application of silver carbonate soon revealed their true nature.

The abnormal condition of the reticulum within the rachitic band was at first regarded as the result of swelling of its fibers. In a recent paper Mallory and Parker¹⁴ have shown that separated collagen fibrils are deeply stained by silver, but compacted fibrils are not. According to these authors reticulum is merely collagen occurring as separated fibrils; when "the fibrils of the reticulum are brought into close apposition through degeneration and disappearance of intervening cells, they no longer stain like reticulum but like collagen." The fact that the marrow in the rachitic band fails to develop normally and that it has relatively few cells strongly suggests that the pale color of the coarse fibers of the reticulum in this area might also be the result of coalescence of collagen fibrils. In order to test this point I stained with anilin blue some sections already stained with silver carbonate. This technique has revealed that the collagen fibers of the normal marrow (Fig. 11, *co*) and the supposedly swollen reticulum take a brilliant blue stain: furthermore, the "swollen" trabeculae of the reticulum are seen to consist of closely packed fibrils. From these facts it seems clear that although absorption of fluid from the marrow spaces may cause a certain amount of swelling in the reticulum this process is far less important than the apposition of considerable numbers of collagen fibrils; and that when this apposition takes place, as pointed out by Mallory and Parker, the compacted fibrils no longer stain like reticulum but like collagen.

The walls of the vessels in rachitic marrow show certain abnormalities that deserve mention. In growing long bones the diaphyseal ends contain slender blood vessels arising from the vascular

network in the marrow of the central cavity of the bone. The vessels run parallel to the axis of the bone, and a number of them reach and cross the zone of proliferation and enter the epiphyseal cartilage where they anastomose with the epiphyseal vessels. The latter enter the cartilage chiefly from its posterior surface and they are already present at hatching time (Fell). The walls of the slender diaphyseal vessels in normal chickens consist of endothelium and an outer adventitial layer formed by delicate collagen fibers enclosing spindle-shaped cells with prominent nuclei.

In the rachitic band the walls of the vessels just described are very thick (Fig. 10, *v*). In hematein-eosin slides the adventitial cells stand out sharply in the midst of the thickened walls. In sections stained with silver carbonate it is easy to see that thickening of the walls of the vessels is due to increase and possibly too to swelling of the adventitial fibers, many of which leave the vessel to enter the reticulum (Fig. 12).

The capillaries of the rachitic marrow are well developed; changes in their walls, similar to those found in larger vessels, are absent. A normal amount of blood is seen in the capillaries.

The condition of the marrow in the rachitic band indicates the presence of an excess of watery fluid in the intertrabecular spaces. This excess has been shown to exist by several of the early pathologists and more recently by Schabad, and Korentchevsky.¹⁵ In addition to the histological aspect of the rachitic marrow there are in some cases other evidences of excessive amount of fluid. Large, irregular cavities are often seen in both the rachitic and normal marrow of rachitic bones; when very large these spaces are visible to the naked eye in gross sections of the bones (Figs. 1 and 2, *l*). Under the microscope the cavities under discussion usually appear empty, through loss of the fluid contained therein after the bones were split for preservation, but there are instances in which they contain a delicate network with a few imprisoned lymphocytes and leucocytes.

The cavities mentioned above possess a definite lining consisting of much flattened cells. My impression is that they are considerably distended lymphatics. The latter are well developed throughout the marrow in the fowl, where they form a loose network with sinusoidal aspect. From this network there arise slender vessels that travel toward the epiphyseal ends of the bone and end blindly be-

tween the strips of cartilage of the zone of provisional calcification. In sections of normal bones the lymph vessels are filled with coagulated plasma containing a few lymphocytes and an occasional leucocyte. In rachitic bones many of the marrow lymphatics are absent, but their place is taken by the large cavities which on account of their distension with fluid cause a characteristic condensation of the osteoblastic layer around the prolongations of cartilage in the zone of provisional calcification (Fig. 8).

In the light of present knowledge on the function of the lymph vessels we may assume that the excess of fluid accumulated in the intercellular spaces of the marrow within the rachitic band is drained by the lymphatics. The wide cavities present in the normal marrow of rachitic chickens represent the larger trunks of the system distended with fluid collected by the branches within the rachitic band.

COMMENT

The descriptions in the preceding pages point to the conclusion that when the antirachitic factor is withheld from partly grown chickens a disorder develops with all the characteristics, both gross and microscopic, of mammalian rickets. Minor differences in the aspect of the avian lesions are undoubtedly due to underlying differences in bone development and structure. In the experiment reported in the present contribution rachitic lesions developed readily but this is probably due to the fact that the chickens had received only enough exposure to sunlight to obtain normal growth; if the birds had been continuously outdoors before the experiment was begun, rachitic changes in the bones might not have been so marked at the end of four weeks.

I quite agree with Pappenheimer and Dunn that leg weakness in chickens deprived of the antirachitic factor during the first weeks of life is not rickets if we regard the characteristic lesions in the bones as the chief diagnostic feature of the disease. As will be shown in another paper rachitic lesions develop very slowly when newly hatched chickens are reared in the absence of direct sunlight, and they are not quite as typical as when the experimental treatment is applied to chickens reared in a normal environment for four or five weeks. But even though the lesions in very young chickens are not clearly of the rachitic type it does not seem advisable to deny alto-

gether the existence of rickets in these birds since rickets is not a disease of the bones but a disorder of nutrition that eventually affects the bones. Furthermore, the evidence gathered from humans shows that rickets is more apt to occur during the second half of the first year and first half of the second year. The absence of rachitic changes in the bones of chicks during the first weeks of life cannot, therefore, be used as a strong argument against the identity of leg weakness with mammalian rickets. On the contrary, it would rather indicate that the causative factors operate in a similar way in animals as different as the mammal and the bird.

In concluding I wish to say that although the formation of abundant osteoid and deficient deposition of phosphorus and calcium in the bones have been repeatedly emphasized by most authors as the outstanding characteristics of rickets, conditions in chickens have revealed other features that may have an important bearing on the production of rachitic lesions. The excessive amount of fluid in the marrow in the rachitic band and distended condition of the lymphatics suggest changes in the permeability of the walls of the growing vessels. It would not be surprising, then, if the deficiency in phosphorous and calcium deposition were due to an altered physico-chemical equilibrium that prevents or hinders passage of one or both of these elements through the young capillary walls, imperfectly developed under the stress of a general disorder of nutrition. In the event of its existence such a mechanism would afford a certain degree of protection inasmuch as it would tend to prevent depletion of the already low blood phosphorus which, although needed for normal growth of the skeleton, is also essential for the fulfilment of other physiological activities. While in mammalian rickets there is no deficiency in the calcium content of the blood, in chickens calcium is low, a fact that may account for the marked enlargement of the parathyroids in birds deprived of the antirachitic factor (Doyle, Nonidez and Goodale).

SUMMARY

1. Lack of the antirachitic factor in chickens previously allowed to grow normally for a period of five weeks leads to a disorder with all the essential characteristics of mammalian rickets.

2. Gross skeletal changes, such as beading and bending of the ribs, deformity of the keel of the sternum and enlargement of the

epiphyseal ends of the long bones were present in several chickens within five weeks after the beginning of the experiment.

3. The bone formed during the experiment appeared under the form of a band placed in long bones between the epiphyseal cartilage and the diaphysis.

4. Microscopic lesions observed in the rachitic band were: (1) excess of osteoid in the spongiosa; (2) active production of periosteal osteoid; (3) increase in thickness of the osteoblastic layer throughout the bone of the rachitic band; (4) abundance of osteoclasts; (5) absence of calcification in the zone of provisional calcification.

5. The marrow of the rachitic band was found to consist chiefly of spindle-shaped cells separated by abundant intercellular fluid, with small groups of myelogenous cells. Capillaries were abundant. That portion of the marrow present in the bones before the beginning of the experiment was practically unaltered, but a few hyperplastic nodules with crowded myelogenous cells were noticed in the vicinity of the rachitic band.

6. Certain peculiarities are described in detail. The most important are: (1) deposition of osteoid fibers without previous erosion of cartilage; (2) intra-osteal formation of giant cells through fusion of hypertrophied osteoblasts; (3) enormous increase in thickness of the reticulum in the marrow of the rachitic band with a corresponding thickening of the walls of the blood vessels; (4) marked distension of the lymphatics.

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DESCRIPTION OF PLATES

PLATE 104

- FIG. 1. Frontal section of the upper end of the tibiotarsus (No. 270, ♂). (*e*) epiphyseal cartilage; (*h*) zone of preparatory calcification; (*l*) lymphatic spaces; (*mc*) endochondral bone with normal marrow; (*p*) zone of proliferation; (*r*) rachitic band. $\times 2$.
- FIG. 2. Longitudinal section through the enlargement of a vertebral rib (No. 255, ♂). Hematein-eosin: (*c'*), (*c''*), lateral masses of cartilage; (*e*) epiphyseal cartilage; (*l*) distended lymphatics; (*o*) periosteal osteoid; (*p*) zone of proliferation of the diaphyseal cartilage. $\times 17$.
- FIG. 3. Active periosteal ossification. The area represented is marked (*o*) in the preceding figure. (*c*) uncalcified cartilage of the lateral mass of cartilage (*c'*) of the preceding figure; (*f*) fibrous layer of the periosteum; (*o*) young osteoid; (*ol*) thickened osteoblastic layer of the periosteum. Hematein-eosin. $\times 107$.
- FIG. 4. Trabecula of young osteoid (*o*) in the tibiotarsus. Silver carbonate method; (*c*) enclosed cartilage cells; (*ol*) osteoblastic layer; (*v*) blood vessel with thickened walls. $\times 266$.
- FIG. 5. Normal femur (No. 254, ♀). osteoid margin (*om*) at the distal tip of one of the strips of cartilage of the zone of provisional calcification. Silver carbonate, gold chloride; (*c*) cartilage (unstained); (*o*) osteoblastic layer. $\times 532$.
- FIG. 6. Rachitic femur (No. 256 ♀). Tip of a strip of cartilage of the zone corresponding to the zone of provisional calcification of the normal bone. Cartilage matrix invaded by osteoid fibers; (*c*) uninvaded cartilage; (*e*) enclosed cartilage cells; (*o*) osteoblastic layer. Silver carbonate gold chloride. $\times 532$.

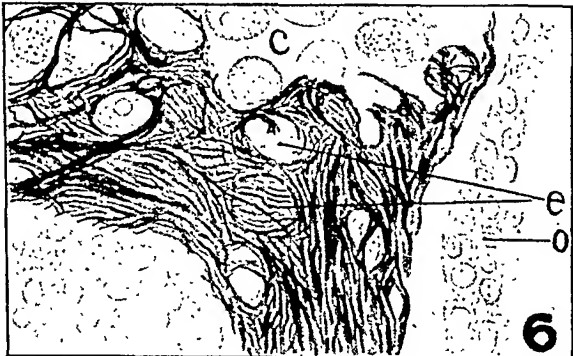
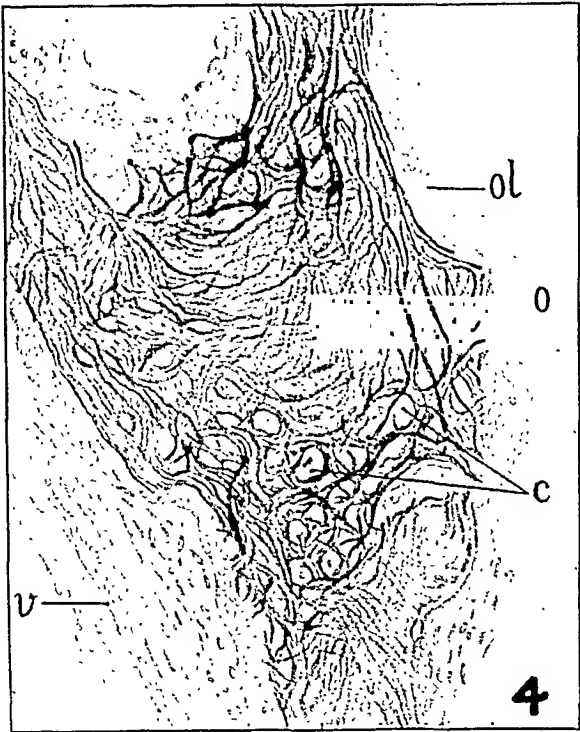
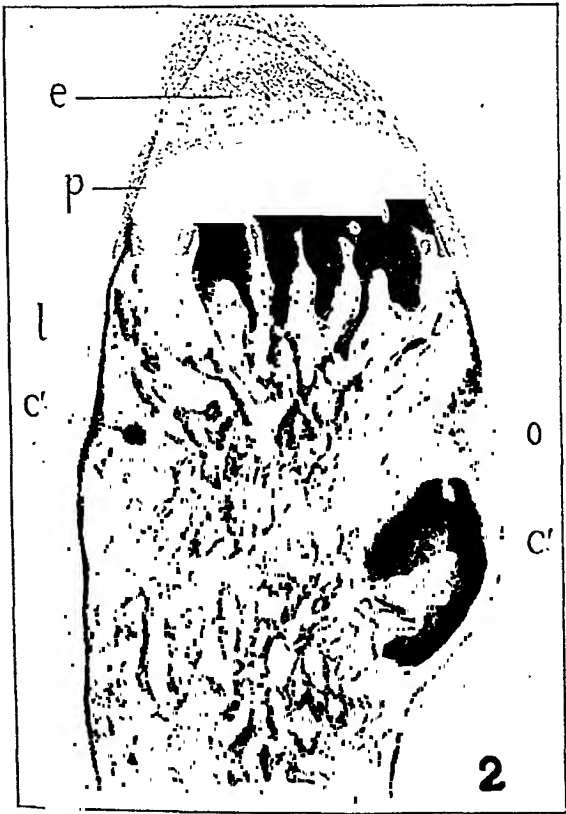
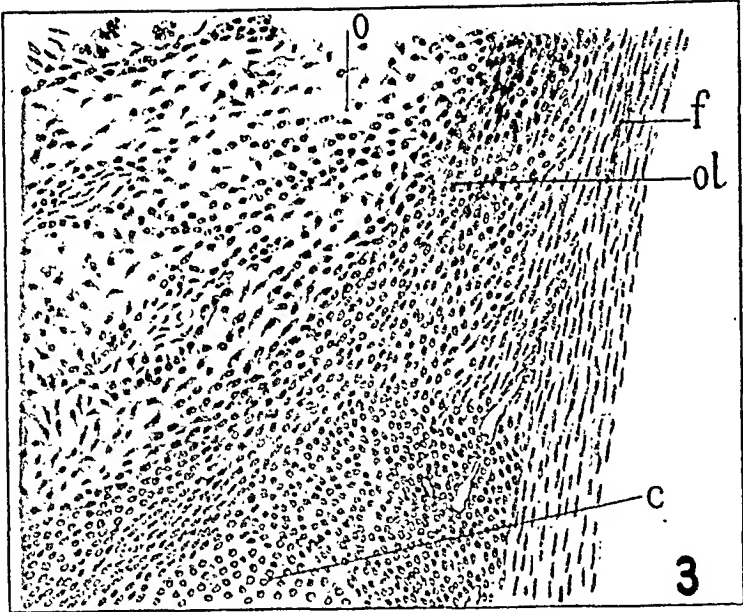
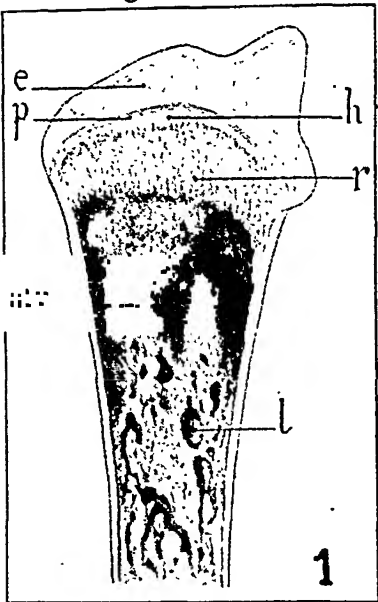


PLATE 105

- FIG. 7. Strip of calcified cartilage from the zone of provisional calcification of the tibiotarsus of a normal chicken (No. 253, ♀). Hematein-eosin; (*c*) cartilage cells; (*g*) giant cells (osteoclasts); (*o*) osteoblastic layer; (*om*) osteoid margin. × 266.
- FIG. 8. Strip of uncalcified cartilage from the same zone of the tibiotarsus of a rachitic chicken (No. 255, ♂). Hematein-eosin; (*m*) marrow cells (myelocytes); (*o'*) hypertrophied osteoblasts. Other letters as in the preceding figure. × 266.
- FIG. 9. Hypertrophied osteoblasts in early stages of their fusion to form intra-osteal multinucleated cells. Hematein-eosin. × 666.
- FIG. 10. Marrow and vessels of the rachitic band of the tibiotarsus (No. 256, ♀) Hematein-eosin; (*g*) osteoclast; (*o*) osteoblastic layer; (*o'*) hypertrophied osteoblasts; (*m*) myelogenous cells; (*v*) diaphyseal vessel. × 266.

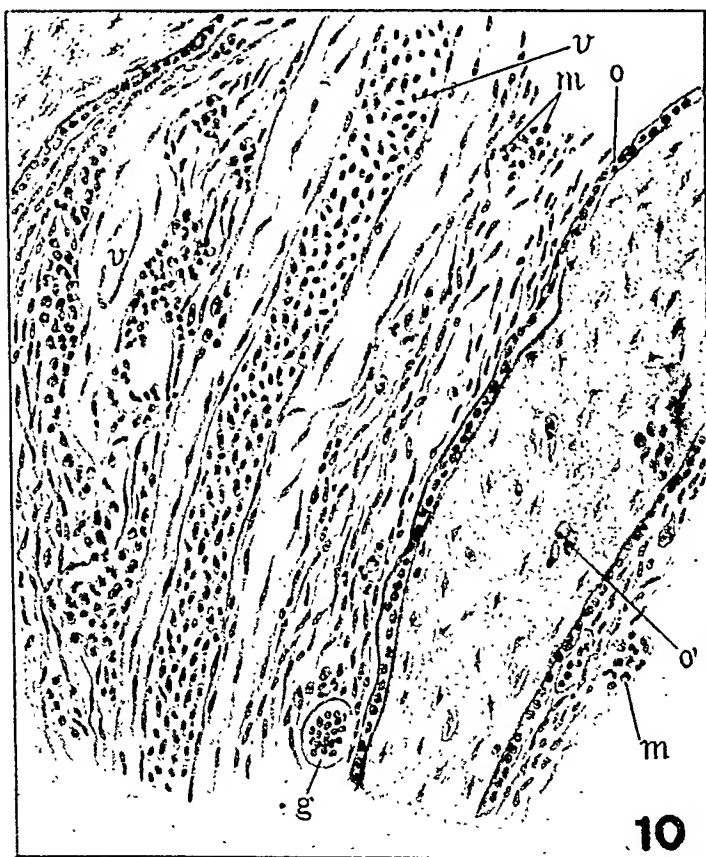
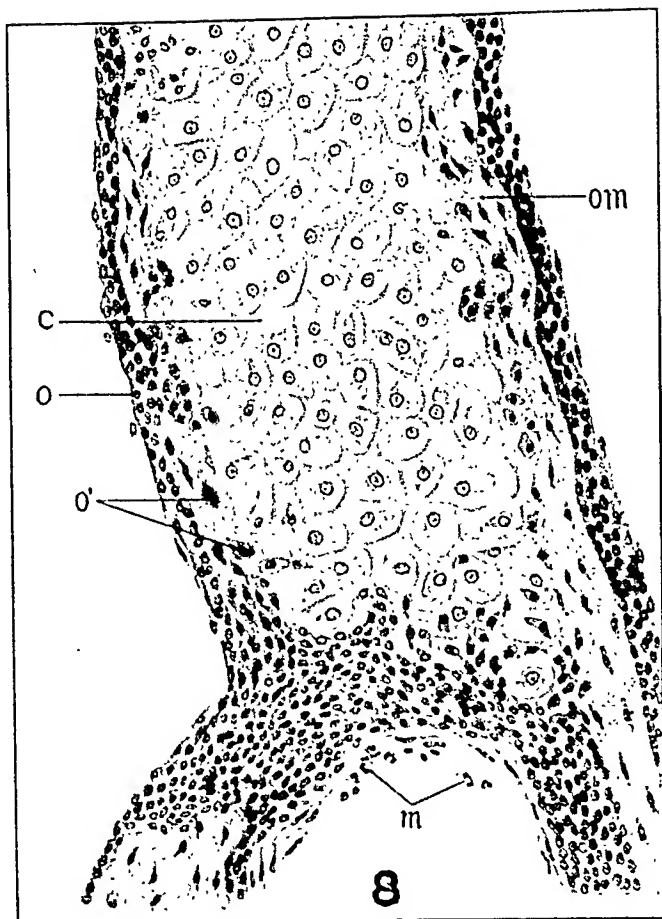
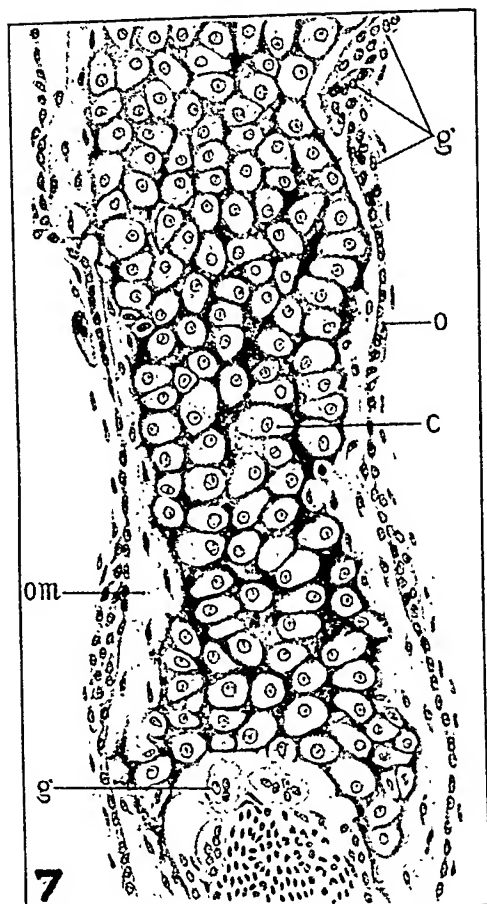
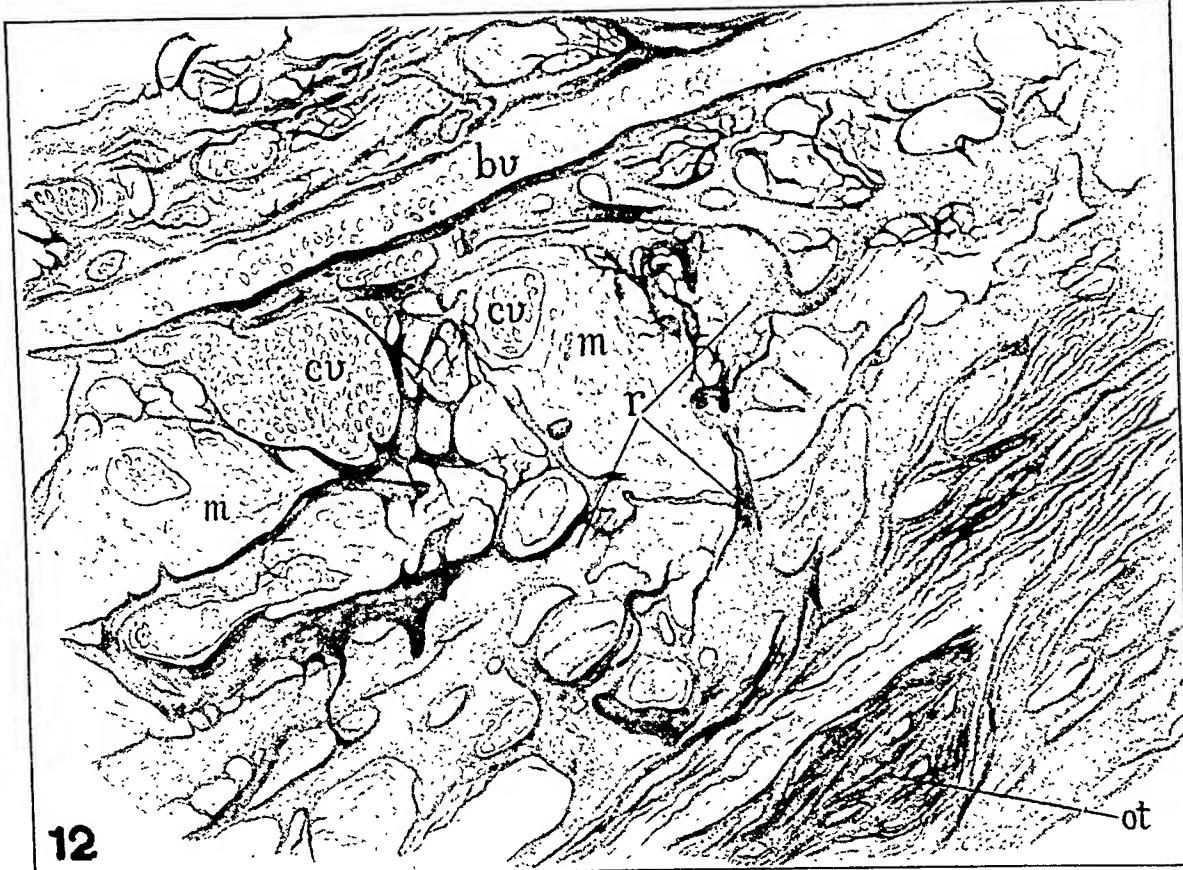
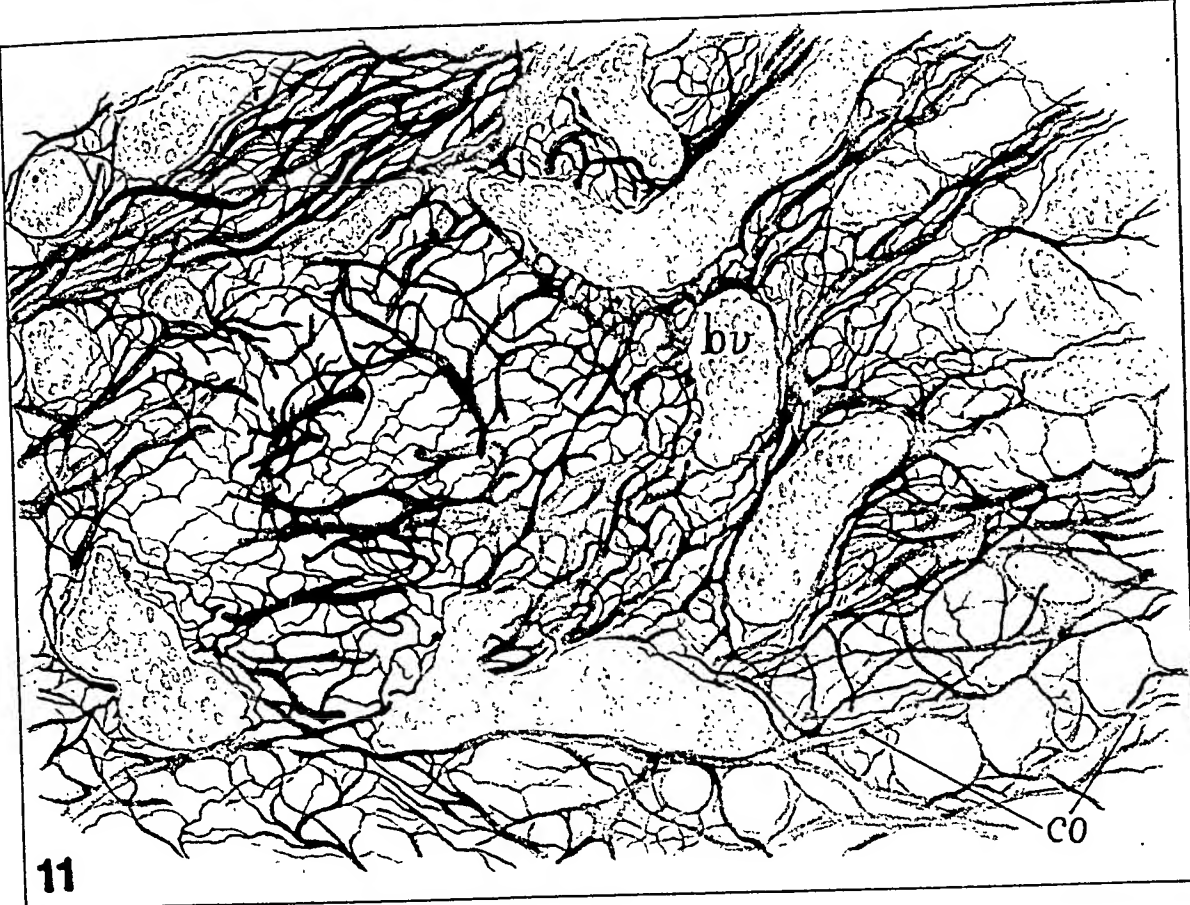


PLATE 106

FIG. 11. Reticulum of normal portion of marrow in the femur of a rachitic chicken (No. 256, ♀). Silver carbonate, toned with gold chloride. (*bv*) blood vessel; (*co*) collagen fibers. $\times 266$.

FIG. 12. Thickened reticulum (*r*) in the rachitic band. Same section and technique as in the preceding figure. (*bv*) longitudinal diaphyseal vessel; (*cv*) capillaries; (*m*) marrow spaces; (*oi*) osteoid trabeculae. $\times 266$.



ABERRANT THYROID GLANDS *

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A review of the literature of thyroid pathology brings out the fact that cases of lateral aberrant thyroid glands are apparently increasing. At the present writing about forty-five cases have been collected from the literature. While the number of cases presented thus far is still comparatively small, we are convinced that the condition is much more common than these reports would suggest. It is probable that there are many other cases which have not been reported, and still others which have been inaccurately diagnosed, as routine histological examinations are not carried out in all hospitals. It is the exceptional case which is diagnosed preoperatively, and, as in our cases, the diagnosis is usually made by the pathologist. The clinical diagnosis is usually "tuberculous glands," "Hodgkin's disease," "lymphosarcoma," "metastatic carcinoma," etc., and without an histological examination such inaccuracies are apt to persist. It is probably the greater tendency for routine pathological examinations of surgical material which accounts for the apparent increase in these cases, as no other obvious factors are to be noted.

It is important to consider this interesting condition, both clinically and pathologically, for several reasons; first, because the developmental side of the question is much discussed and far from being settled; second, because its frequent occurrence and pathology establish it as a clinical entity of note; third, its tendency toward malignant degeneration becomes of serious import to the patient; and fourth, because these glands may be greatly influenced by the changes in the normal and pathological thyroid gland itself.

EMBRYOLOGY AND COMPARATIVE ANATOMY

The development of the thyroid gland is one of the most disputed problems embryologically. It was felt that a study of these anomalously placed bits of thyroid tissue might be of some value in its solution.

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The older embryologists (His, Born, Prenant and others) concluded that the thyroid has a dual origin. They believed that the major portion of the gland, including the isthmus, is derived from a median down-growth of epithelium from the floor of the pharynx. This occasionally persists as the thyroglossal duct. The remainder of the gland they considered as arising from the lateral outpocketings of the pharynx which normally fused with the median portion. On this basis it would be simple to explain the presence of aberrant glands as due to a failure of the lateral portion to fuse with the median.

We have since come to accept the fact that in man the third and fourth branchial clefts give rise to small masses of epithelial cells which migrate toward the thyroid and eventually come to rest, usually at its anterior and posterior borders. These are the parathyroid glands, which in postnatal life are usually cords of cells, but may develop lumina in which accumulates a pale staining colloid-like material. This phase of parathyroid physiology is one which has not perhaps been generally recognized and has given rise to some of the confusion concerning the aberrant thyroid glands, as they have many points of similarity under the microscope. The parathyroids in man vary considerably in both number and position, but are usually paired and four in number. It may be said that anatomists today are generally in accord regarding these two branchial pouches.

Following the discovery of the parathyroid glands, it was believed that the thyroid was developed entirely from the median anlage. Subsequent investigation, however, revealed a developmental relationship between the "fifth" pouch and the thyroid gland. The textbooks give opposing views on this subject and we find little agreement concerning the existence of such a pouch.

Kingsbury¹ studied a series of embryos and came to the conclusion that there is no reason for considering that the ultimobranchial body represents any specific fifth pouch, but is merely formed by continued growth activity of the posterior portion of the pharyngeal complex. In the mechanics of growth, by the shiftings that occur, this posterior portion becomes fused with the thyroid gland. He also concludes that "it soon, however, becomes indistinguishable and apparently finally in man disappears (typically) without a trace."

The work of Badertscher^{2,3} confirms Kingsbury's investigation and goes a step further. He studied a large series of pig embryos in

all stages of development from the 15 mm. pig to the 270 mm. pig (full term). Then he studied pigs that were a few hours old, one 7½ days old, one 15 days old, one 28 days old, one 42 days old, one 56 days old and in addition several adults. He was able to identify and follow the development and fate of the ultimobranchial bodies through the entire series. He finds that these bodies participate in the formation of the thyroid gland. He states that, "In conclusion, it can be said that since the ultimobranchial bodies fuse with the thyroid gland and also form colloid, the boundary between these structures and the gland becomes obliterated, so that it is impossible to determine the exact relative proportion that is contributed to the thyroid gland by the ultimobranchial bodies and the median thyroid anlage. Owing to the variable developmental behavior of the ultimobranchial bodies, the relative proportion they contribute to the thyroid gland undoubtedly varies in different pigs. It is, however, quite evident that only a relatively small portion of the gland is derived from the ultimobranchial bodies."

Grosser in Keibel and Mall's textbook ⁴ in referring to the epithelial bodies which develop from these various pharyngeal out-pocketings calls attention to the fact that there is considerable variation in development and striking anomalies of position as well as diminution and increase in numbers. It would seem possible then to account for the presence of these lateral aberrant glands as arising from cells in the posterior portion of the pharynx which in migration have failed to fuse with the median thyroid anlage in its descent. In accordance with Virchow's theory of "fetal rests" these cells may subsequently give rise to tumor formation, when they are derived from third or fourth pouch cells, to the so-called "parastruma" and from the fifth pouch to the true aberrant thyroid. The relatively constant type of degenerative lesion is believed to be more than accidental and suggests that these aberrant thyroid glands may have such an embryological origin.

In a series of cases collected from the literature by Billings and Paul ⁵ they note that 70 per cent with a diagnosis of neoplasm had tumors of the papillary type. To that list is added our series of four cases, all with the diagnosis of papillary cystadenoma or papillary adenocarcinoma.

Neoplasm of median or lingual thyroid is very rare. We have not seen a single case, and can agree with these authors "that there is an

inherent difference in the lateral thyroids as opposed to the median in their tendency toward a specific type of tumor formation." They also recall the dual nature of the pancreas and note that while carcinoma of the acini is not uncommon, carcinoma of the islands is comparatively rare. This is an interesting analogy.

That the thyroid in its development is constantly associated with the migration and fusion of epithelial cells derived from the so-called fifth pouch or posterior outpocketing of the pharyngeal complex (the ultimobranchial body); that these epithelial masses may vary considerably in number and position; that the lateral aberrant thyroid glands tend in a high percentage of cases to undergo papilliferous and cystic degenerative change, a lesion never associated with lingual thyroid tissue; these facts, it would seem, serve to identify these tumors as a specific group, and to account for their origin on sound embryologic evidence.

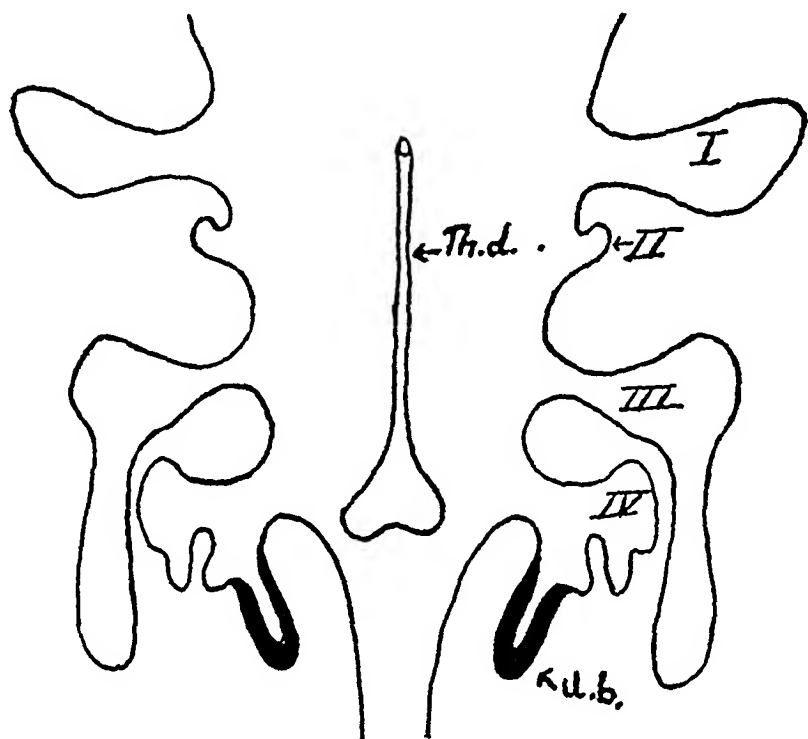
CLINICAL DISCUSSION

In a series of nearly four thousand cases of thyroid disease seen in the Lahey Clinic only four with lateral aberrant thyroid tumors were encountered, an incidence of only 0.1 per cent. These patients came, not because of thyroid disease, but were alarmed by a slowly growing tumor in the side of the neck. These tumors varied in size, shape and position. They were firm, non-fluctuant, non-tender and gave no subjective symptoms. The duration was from several months to several years. They occurred either as a large single mass or as a chain of gland-like enlargements in the neck along the course of the internal jugular vein. The single type has been as a rule, in our experience, low in the neck, posterior and lateral to the sternomastoid muscle. There are no clinical features other than the location which might aid in diagnosis, and in our four cases the diagnosis has only been suspected once. The importance to the patient, because of the prognosis as compared to that of Hodgkin's disease, for example, cannot be overemphasized, and its consideration in differential diagnosis in lateral tumors of the neck should not be overlooked.

Surgically these tumors should be treated by as complete removal as is possible in each case. It is unnecessary to emphasize the obvious fact that early removal of all suspicious masses in the neck should be strongly urged. No new or special problems of surgical technique

have been encountered in these cases. All have made good recoveries from their operations and thus far no case has had any evidence of recurrence of the disease. The time elapsed since operation has of course been very brief in two of the cases.

We believe that X-ray treatment should be advised when papillary cystadenoma has been found at operation. This type of thyroid



TEXT-FIG. 1. Schematic drawing of the pharyngeal pouches of an early human embryo. I, II, III, IV, lateral outpocketings. *Th. d.*, thyroglossal duct. *U. b.*, ultimobranchial body—posterior continuation of pharynx (Groschuff and Kohn in "Keibel and Mall").

tumor responds most satisfactorily to X-ray treatment and we favor the use of X-ray after operation whether or not the tumor appears to be malignant microscopically. We likewise believe that post-operative X-ray treatment is advisable when multiple areas of aberrant thyroid tissue growth are encountered because of the possibility that malignant changes may already have occurred.

CASE REPORTS

CASE 1. Clinical History: N. E. D. H. 30,755. Mrs. B. H., aged 70, entered the hospital July 7, 1925. The chief complaint was a mass in the left side of the neck of 3 years duration. The family and past history are unessential.

Present Illness: Began 3 years ago, when the patient noticed a mass the size of a hen's egg in the supraclavicular space. This has continued to grow upward and posteriorly. It has never been painful or inflamed. X-ray of the chest is negative.

Provisional Diagnosis: Tuberculosis? Hodgkin's disease? Sarcoma?

Operation: July 8, 1925. In the left supraclavicular space was a mass the size of an orange. Transverse incision made over the mass. The sternomastoid was retracted medially. The mass was found adherent to the surrounding structures including the internal jugular vein. It was carefully dissected away and removed. The mass had the appearance of malignancy. The operative diagnosis was (?) of malignant lymphoma of the left neck.

Pathologist's Report: DS-25-1039. The specimen consists of a large mass about 9 cm. in diameter. It is rounded and rather coarsely lobulated on the exterior. The cut surface reveals the presence of a coarse fibrous trabeculation, between which is a more or less dark, mushy material which appears somewhat granular, is reddish gray in color and extremely friable. There is a suggestion of a papilliferous type of lesion with extensive secondary hemorrhage and cystic degeneration (Figs. 1 and 2).

Microscopic: The specimen appears to be a papillary adenocystoma of thyroid tissue. There are areas where the cells are so closely packed together that the papillary arrangement is somewhat lost, but most of the specimen presents this feature very markedly. Occasional mitotic figures are found and the cells are arranged with a good deal of regularity. There is no definite colloid. The epithelium is nearly all high columnar in type. Some evidence of inflammatory infiltration is found with foci of polymorphonuclear cells. This is a type of tumor which, while not yet histologically definitely malignant, tends to undergo malignant degeneration with subsequent metastasis so that the prognosis should be extremely guarded and the case treated as potentially malignant.

Diagnosis: Papillary cystadenoma (aberrant thyroid).

CASE 2. Clinical History: N. E. D. H. 37,550. Miss E. T., aged 20, entered the hospital November 25, 1926, with a mass of glands posterior to the lower end of the right sternomastoid muscle. The family and past history are unessential.

Present Illness: Recently the patient noticed a mass of glands posterior to the lower end of the sternomastoid muscle in the supraclavicular space. These masses were not tender, did not fluctuate in size, and gave no subjective symptoms. No glands could be felt elsewhere in the body. She had lost 20 lbs. in weight in the past two years. Physical examination was negative except for local.

Preoperative Diagnosis: Tuberculosis of cervical lymph nodes.

Operation: November 24, 1926. A gland about the size of a walnut was removed from the lower portion of the sternomastoid. On section this gland closely resembled aberrant thyroid tissue.

Pathologist's Report: DS-26-2331. The specimen consists of an encapsulated tumor mass measuring about 2 cm. in diameter and roughly spherical. It is purplish red in color and is covered with a serosanguinous fluid. The cut surface consists of soft, friable semi-translucent, pink tissue which tears easily and is finely papilliferous.

Microscopic: It presents a papillary epithelial hyperplasia with some tendency to alveolar formation. There is a very fine loose connective tissue stroma which takes no active part in the proliferation. In various portions there are typical alveoli with some tendency to colloid formation. In several places the hyperplastic tissue is attempting to break through the capsule. Mitotic figures are not in evidence. Histologically the specimen is still benign but it may very likely recur (Figs. 3 and 4).

Diagnosis: Papillary cystadenoma (aberrant thyroid).

CASE 3. *Clinical History:* N. E. B. H. 24,149. Dr. F. W., aged 67. Entered the hospital Aug. 4, 1927, with tumor of the right side of the neck; duration 20 years. The family history and past history are unessential.

Present Illness: The patient has had the tumor for 20 years. It has remained relatively constant in size, shape and consistency. It has never been tender or fluctuant, and has not given any subjective symptoms. There has been no tremor, palpitation or pressure. There has been no loss of weight.

Physical Examination: The physical examination was unimportant except for the local, which showed a fairly large tumor mass deep to the lower portion of the right sternomastoid muscle. An X-ray examination previous to entry showed compression of the trachea from both sides opposite the seventh cervical vertebra. The anteroposterior view showed no abnormalities.

Diagnosis: Large adenoma of the right lobe of the thyroid extending into the thorax and deviating the trachea to the left.

Operation: August 6, 1927. Outside the thyroid body, underlying the sternomastoid muscle, there was a large adherent mass containing a yellow mucoid fluid, and measuring 8 by 4 by 5 cm. There was a similar nodule about 2 cm. in diameter in the lower right lobe of the thyroid. They had no connection. There was some suggestion of malignancy.

Pathologist's Report: DS-27-2010. The specimen consists of two portions of thyroid tissue. The smaller portion is about the size of a large marble and has been dissected from the lower right pole of the thyroid. It is firm on palpation and weighs 12 gm. The cut surface shows the tumor to be well encapsulated. The center of the tumor is made up of a yellowish brown material, granular and fri-

able. From the wall of the adenoma areas of proliferating papilliferous tissue are seen. The second portion of the tissue lay 4 cm. away from the thyroid gland, lateral to it and under the sternomastoid muscle. This mass measures 7 by 4 by 2 cm. and weighs 38 gm. It is well encapsulated. The cut surface presents the same picture as that described for the adenoma.

Microscopic: The sections through the lower pole tumor and the aberrant portion of the gland present an identical histological picture of a papillary cystadenoma. Both appear well encapsulated and show no invasion of the capsule. The epithelium is arranged in the form of papillary projections which have a core of rather dense connective tissue stroma. There is some tendency toward alveolar formation and colloid retention. Some cystic degeneration is seen and a rare mitotic figure. There is considerable arteriosclerosis of the smaller blood vessels (Figs. 5 and 6).

Diagnosis: Multiple papillary cystadenoma (aberrant thyroid).

CASE 4. Clinical History: N. E. D. H. 41,390. Miss F. K., aged 21, entered the hospital September 14, 1927, with enlarged glands on both sides of the neck of several months duration. The family history and past history are unessential.

Present Illness: About six months ago the patient noticed a chain of enlarged glands on the right side of the neck along each side of the sternomastoid muscle. The glands have gradually increased in size until the patient feels that her head is pushed to the opposite side. Three months ago a similar chain of glands appeared on the left side. These also have been increasing in size. There was no pain or tenderness over the affected area. There has been no loss in weight and no attacks of sore throat. There was no history of tuberculosis.

Physical Examination: The physical examination was negative except for local. Numerous glands were found on each side of the neck, extending from the mastoid region to the clavicle. They were discrete, not indurated and in no way adherent. They were quite characteristic of Hodgkin's disease. No glands were found in the groin or axillae. The spleen was not enlarged and no mesenteric glands were found.

Biopsy: September 15, 1927. A gland about the size of a lime was removed from the supraclavicular region.

Pathologist's Report: DS-27-2345. The gross specimen consists of several lymph glands which are bound together by adipose and connective tissue. They are pinkish white in color and have a semi-solid consistency. There are several cysts which range in size from that of a pea to a small marble and contain a blood-tinged fluid. Microscopically the sections present a picture of a papillary cystadenoma. The villus processes are covered with a columnar epithelium which rests on a connective tissue stroma. Many fields show considerable calcification. The colloid material is moderate in

amount, and many alveoli are seen. A few mitotic figures are seen suggesting possible beginning malignant degeneration.

Diagnosis: Papillary cystadenoma of the thyroid (aberrant); (?) malignancy.

On the basis of the pathological findings operative measures were advised and on September 21, 1927, a complete dissection of the right side of the neck was made. Thyroid tissue was found extending into the chest below, and as high as the submaxillary gland above. It was adherent to the sternomastoid muscle and the internal jugular vein. All portions that were grossly involved were removed. A portion of the thyroid was removed for diagnosis. There was no connection between the gland and the aberrant tissue in the neck.

Supplementary Pathological Report: DS-27-2404. The specimen consists of a mass of glands which are matted together with connective tissue. The total weight is 70 gm. They are very red in color and rather firm in consistency. On section they appear to be fairly well encapsulated and present a picture of aberrant thyroid tissue. There are seen small cysts which contain a brownish-colored fluid and old blood. The gross appearance is not that of tuberculosis or of Hodgkin's disease. One of the glands shows some calcification. Two other small pieces of tissue have been submitted for examination; one is a portion of the internal jugular vein and the other is a lymph gland.

Microscopic: The sections microscopically present a typical picture of a papillary adenocarcinoma. The lymph glands show extensive metastases. However, no invasion is seen of the jugular vein. The epithelium is columnar in type and it rests on a connective tissue stroma which in places shows considerable calcification. The same type of lesion is seen in several of the lymph nodes (Figs. 7 and 8).

Diagnosis: Papillary cystadenoma (aberrant thyroid) with metastases to regional lymph nodes.

The patient made an uneventful recovery and was discharged at the end of two weeks to have subsequent X-ray treatments.

DISCUSSION

Little needs to be added to the data which have been submitted. There is a point of some speculative interest, however, which might be called to attention. In a study of the malignant tumors of the

thyroid gland which we are in process of making, the extraordinary similarity between most of the papilliferous adenomata and these tumors of lateral aberrant thyroid tissue has been constantly noted. This is true not only morphologically, but clinically in many respects. It is in this group of tumors that the prognosis is relatively good. They are slowly growing tumors which tend to spread by direct lymphatic extension, first to the regional lymph nodes. The possibility that this entire group of tumors may arise from these same fifth pouch pharyngeal epithelial rests, in the one case representing displaced cell nests in the course of migration; in the other, displaced cell nests in the process of fusion of the lateral and median portions, is one which it is hard to overlook. It is a theory which does not lend itself readily to proof, but the evidence at hand points strongly in such a direction. This by no means excludes the possibility of a parenchymatous origin for certain of these tumors, but it certainly offers an attractive hypothesis for the explanation of the great majority of them. The proof can be found only in the examination of a sufficient number of cases and the finding of a series along the entire migratory course of the epithelial outgrowth from the posterior pharynx to the thyroid itself. In our series, one of the cases (Case 3) suggests such a possibility; two tumors of identical histology; one within the lower pole of the gland, the other 4 cm. removed.

Pathologically, likewise, little can be added beyond a more detailed discussion of the histological findings. The outstanding features may be cited as the distinct adenomatous character of the tumors with the presence of a well developed capsule; the evidence of chronicity and slow development of the tumors with inevitable calcification, hemorrhage, hyalinization of the stroma and cystic degeneration; the uniform papilliferous arrangement of the cells; and their tendency toward lymphatic extension, as contrasted to the group of solid adenomata (fetal type) which invariably invade the blood stream when they become malignant, as Graham ⁶ has so succinctly pointed out.

For the sake of completeness it may perhaps not be out of place to call attention to the occasional occurrence of aberrant thyroid tissue in certain of the teratoid tumors; notably, of the ovary. A considerable literature has accumulated during the past few years on this group of cases.

Kovacs⁷ recently described a rare tumor of the ovary, goiter-like in nature. Symptoms of exophthalmic goiter had developed with the ovarian tumor and subsided with its removal. The structure of the ovarian tumor was that of a colloid goiter and it seemed not only morphologically and chemically, but functionally, true thyroid tissue.

We have seen one case of aberrant thyroid tissue in an ovarian tumor. The patient, Mrs. B. E. S., aged 40, was admitted to the hospital March 13, 1927. The chief complaint was belching of gas and abdominal distention. For the past six months she had noticed irregular and profuse flowing. The physical examination was entirely negative except for a multiple fibroid uterus. Through a median suprapubic incision a large multiple fibroid uterus was removed together with a multilocular teratoid cyst of the right ovary. The left tube and ovary were left behind. Pathologically the endometrium showed a chronic hyperplastic endometritis, while the leiomyomata presented the usual appearance. Microscopically the cystic ovary showed hair follicles, sebaceous glands and a fairly large area of thyroid tissue characterized by acini lined with cuboidal epithelium and containing colloid material. Thyroid tissue is comparatively rarely seen in these tumors. The patient showed no symptoms referable to an abnormal thyroid physiology.

SUMMARY

Four cases of lateral aberrant thyroid gland tumors are presented, including the clinical and pathological findings.

Their probable etiology is discussed. It is believed that these tumors arise in cell masses (the ultimobranchial bodies) which develop from the posterior portion of the pharyngeal complex, the so-called fifth pouch. This is not a true pouch, but a projection backwards and downwards of the posterior portion of the pharynx. These cells in their migration may fail to meet and fuse with the thyroid and give rise subsequently to tumor formation.

Attention is called to the possible relationship of the papilliferous tumor of the thyroid gland itself to these same cell rests after they have become incorporated in the gland.

Emphasis is placed on the importance of diagnosis in these cases because of their potential malignancy, the difficulty of preoperative differential diagnosis, and their relatively favorable prognosis.

Postoperative X-ray treatment is recommended for all cases.

A case of aberrant thyroid tissue in a multilocular teratoid cyst of the ovary is described.

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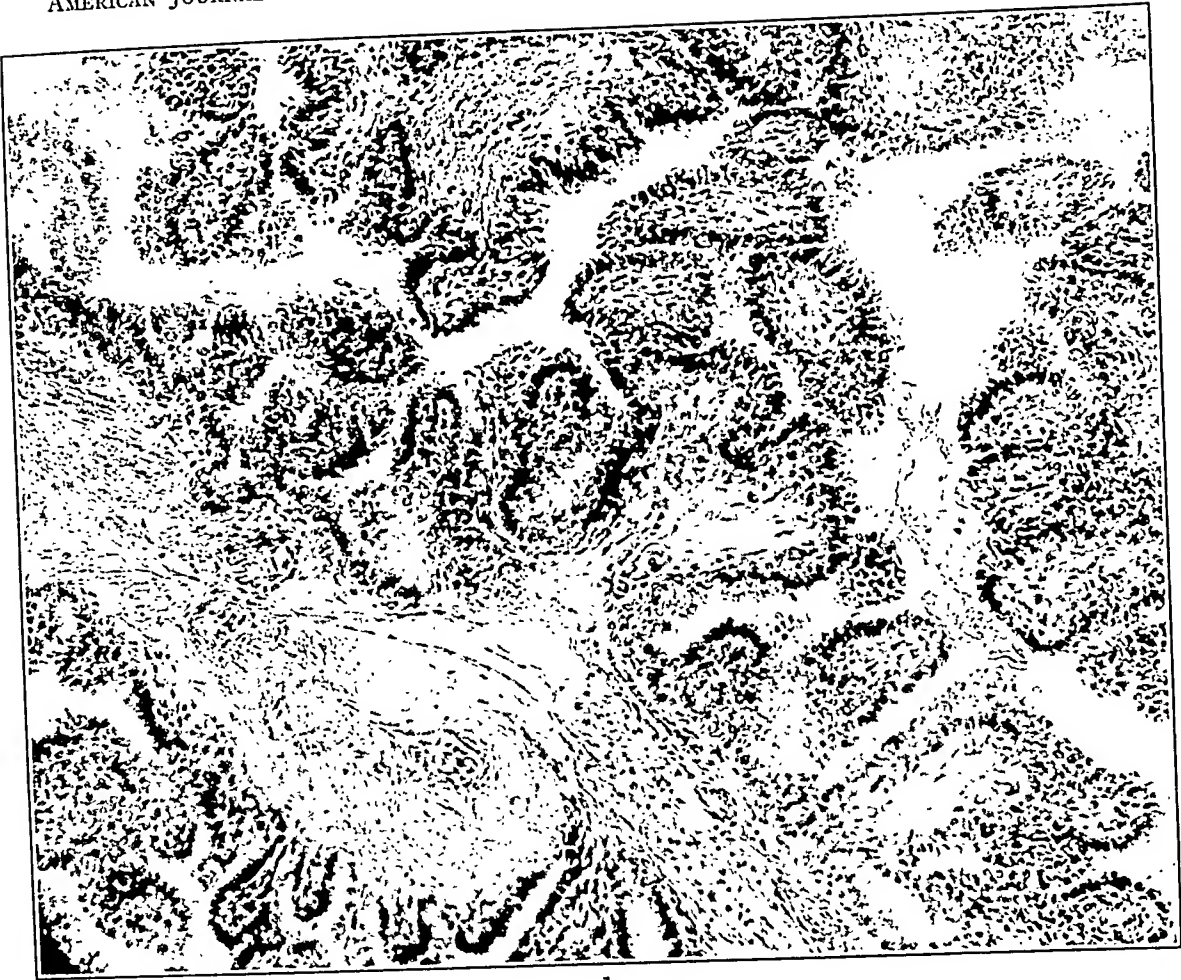
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DESCRIPTION OF PLATES

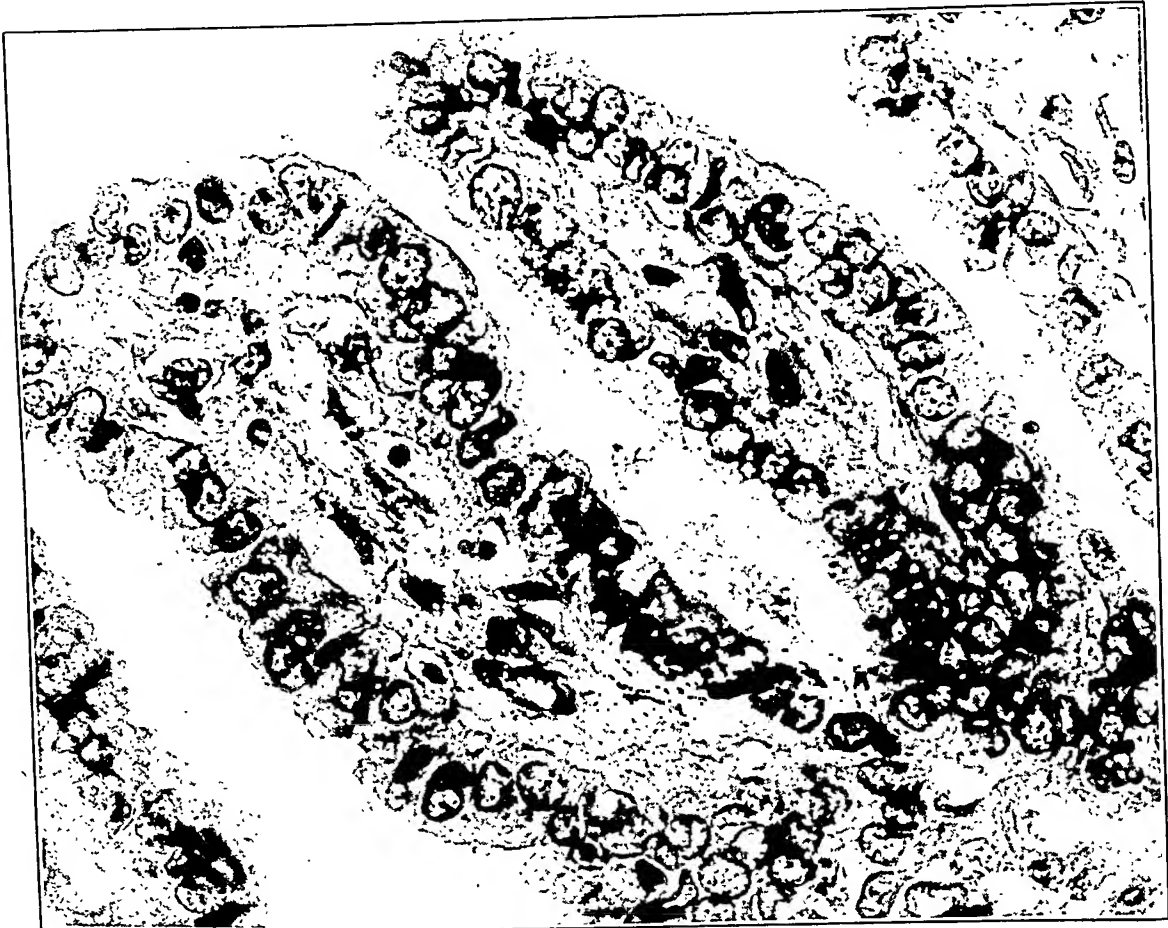
PLATE 107

FIG. 1. Low power photomicrograph. Illustrates the papillary hyperplasia. (Case No. DS-25-1039.)

FIG. 2. Same as Fig. 1, high power, showing the arrangement of the cells on papillary stalks.



1



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PLATE 108

FIG. 3. Illustrates the relatively benign appearance of these tumors in their earlier stages. Note the marked papillary hyperplasia comparable to that seen in the papillary cyst adenomata of the ovary. (Case No. DS-26-2331.)

FIG. 4. Same as Fig. 3.



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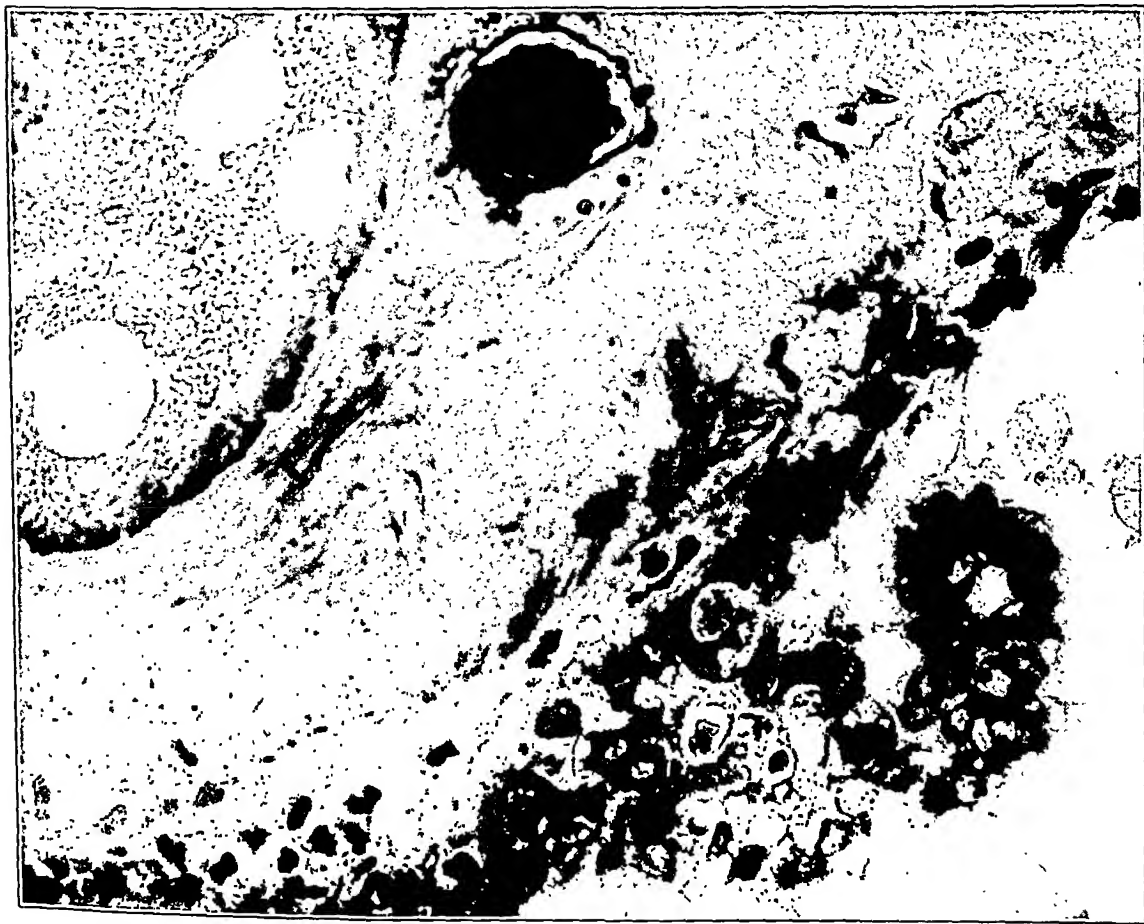
PLATE 109

FIG. 5. Low power photomicrograph of an area through the wall of the tumor. This emphasizes the long duration of certain of these cases with marked fatty degeneration and calcification. (Case No. DS-27-2010.)

FIG. 6. High power, same as Fig. 5.



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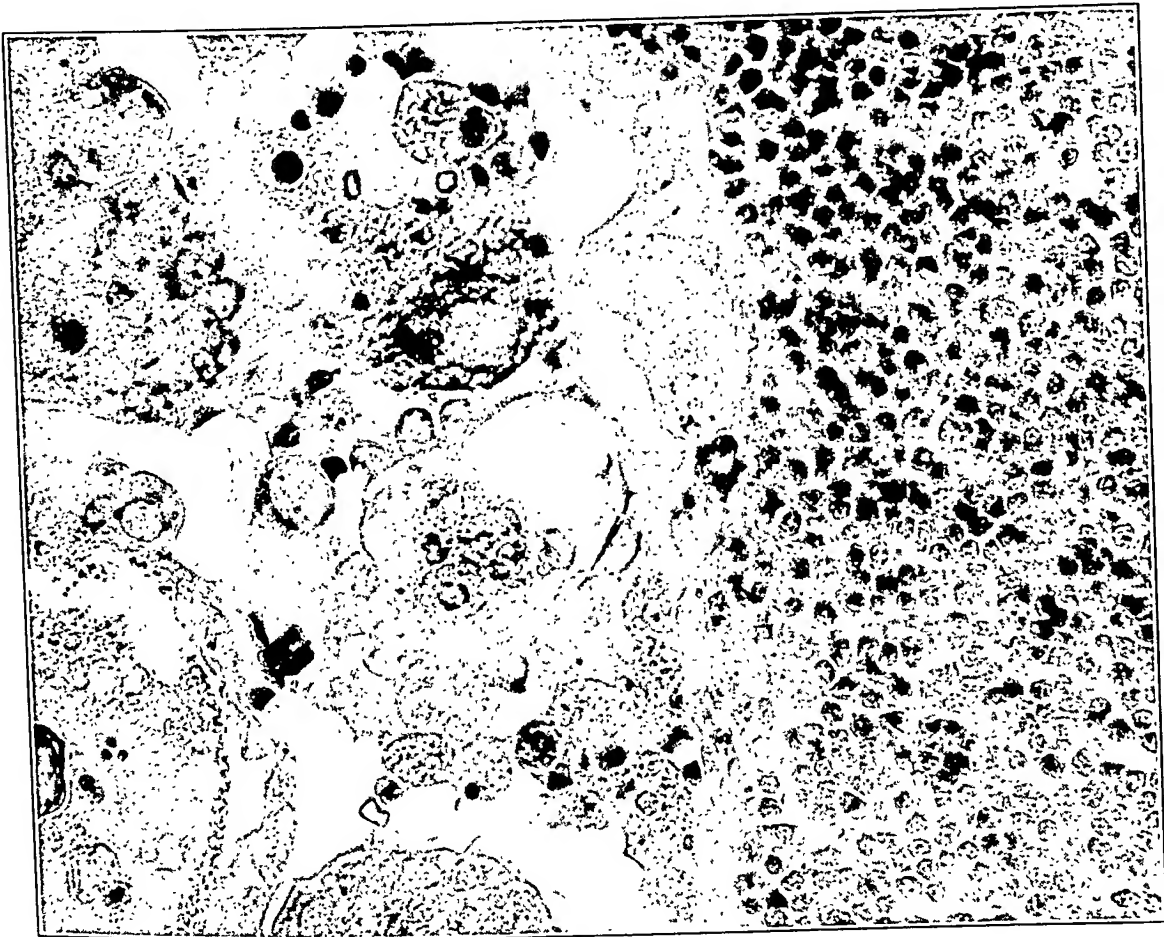
PLATE 110

FIG. 7. Low power photomicrograph illustrating the invasion of the regional lymph nodes of the neck by tumor tissue. The slow rate of growth is emphasized by the papillary proliferation, cystic degeneration and absence of mitotic figures. (Case No. DS-27-2345.)

FIG. 8. High power, same as Fig. 7.



7



8

STAINING FIBRILLARY NEUROGLIA IN FORMALIN-FIXED MATERIAL *

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Mallory's¹ excellent phosphotungstic acid hematoxylin method for neuroglia is applicable only to Zenker-fixed material. Bailey's² more selective stain for fibrillary neuroglia with neutral ethyl violet-orange G was also developed specifically for material preserved in this fixative. This leaves for formalin-fixed material practically only the method of Weigert which, in addition to being an involved procedure and requiring expensive chemicals that are often difficult to obtain, gives blurred and indifferent pictures on material fixed in formalin for long periods before mordanting.

I have tried refixing formalin tissue in Zenker's solution but the results on such material with the methods either of Mallory or of Bailey are not worth the effort.

For many years Cajal and Del Rio Hortega have advised the use of ammonia for removing an excess of formalin from sections for various silver stains. Recently Globus³ has applied this principle to the staining of material fixed in formalin alone with the Spanish silver methods which originally required fixation in formalin and ammonium bromide. He treats frozen sections cut from formalin-fixed blocks with ammonia overnight in a warm oven and then "bromurates" them with hydrobromic acid. Upon sections thus treated he applies the various silver techniques with excellent results.

This being the case, I have treated formalin-fixed blocks with ammonia to remove the formalin, refixed them in Zenker's solution and stained sections cut from paraffin blocks by the methods of Mallory and of Bailey in order to stain the neuroglia fibrillae. The procedure is as follows:

(1) Blocks of tissue 2 or 3 mm. thick are cut from the formalin-fixed brain, cord, or tumor, and placed in a dish filled with about 100 cc. of distilled water containing 30 to 40 drops of strong ammonia water. This is kept air-tight in an oven at 37° C for 4 days.

(2) The blocks are washed for 12 to 24 hours in running water.

(3) They are then fixed in Zenker's solution for 24 hours.

* Received for publication May 18, 1928.

(4) Embedding in paraffin, cutting and staining are then carried out as described by Mallory or Bailey, to whose work the reader is referred.

The results obtained by this procedure are exceedingly satisfactory. Within limits of fifteen to twenty years, the length of time that material has been fixed in formalin does not seem to affect the quality of the stain. The phosphotungstic acid hematoxylin stain brings out the cytoplasm of the protoplasmic neuroglia as well as the fibrillary neuroglia and fibrillae, whereas the ethyl violet-orange G method stains the fibrillae more specifically as well as the fibrillary astrocytes. The latter stain, moreover, does not stain myelin sheaths or connective tissue as the Mallory procedure is likely to do.

Unfortunately the difficulty in preparing the neutral ethyl violet-orange G stain has prevented its general use. This difficulty, however, has been completely overcome by Dr. Bailey, who is permitting me to publish his present simple method of preparation of his stain, which is as follows:

- (1) Mix ethyl violet 1.0 gm. } accurately
Orange-G 0.5 } weighed
- (2) Add 100 cc. distilled water and stir thoroughly.
- (3) Place in or on a warm oven (37°C) for 12 to 24 hours to precipitate.
- (4) Decant supernatant fluid and wash precipitate several times with distilled water.
- (5) Place in oven to dry.
- (6) Make a saturated solution of dried precipitate in absolute alcohol.

This solution, if well stoppered, will keep indefinitely.

For staining, use one part stock solution to three parts of 20 per cent alcohol.

One difficulty may arise from the fact that not enough material is on hand at times to permit the assignment of a whole block for this purpose. In fact, it may happen that all the tissue available consists of a single formalin-fixed block which is already embedded in paraffin. In this case, too, it is possible to obtain a very satisfactory re-fixation as follows:

- (1) Sections* are deparaffinated and placed in 50 cc. of water containing 10 drops of ammonia for 24 hours at room temperature.

* Sections to be treated with strong alkalis are less likely to float off the slide if, instead of egg-albumin and glycerin, gelatin is used to make them stick on. For this purpose 2 square inches of pure gelatin are dissolved in 250 cc. of boiling water. The sections are floated on the surface of this solution while still quite warm, and drawn up on clean slides. The excess moisture is wiped off and the slides put for 24 hours into a warm oven (37°C) in which a beaker of formalin stands open.

- (2) The ammonia is washed out with a slow stream of running water for 1 to 2 hours.
- (3) Sections are refixed in Zenker's solution for 12 to 24 hours,
- (4) Washed in running water 3 to 4 hours,
- (5) Treated with iodine solution to remove the bichloride crystals and stained.

The resulting stain is not so deep or so sharp as when the tissue is treated in block before embedding, but is still remarkably fine. It is not unlikely that equally good results as from refixation in block could be obtained if the sections were better ammoniated, but such a procedure only serves to float off the section from the slide and ruins the preparation.

Comment: By this method, it is possible to obtain excellent preparations to show neuroglia fibrillae in material which, owing to its long fixation in formalin, does not yield itself to the satisfactory demonstration of these elements by any other means. The importance of this is evident when it is recalled that for numerous decades formalin fixation, especially for the central nervous system, has been not only the favorite, but, in many instances, the sole preservative employed.

In dealing particularly with the gliomatous tumors of the nervous system where a reliable and specific method for the demonstration of glia fibrillae is often essential for their classification, this method may serve to save much valuable material which might otherwise be wholly useless, or at least unsuited for thorough investigation in the light of recent developments in our knowledge of these neoplasms.

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DESCRIPTION OF PLATE

PLATE III

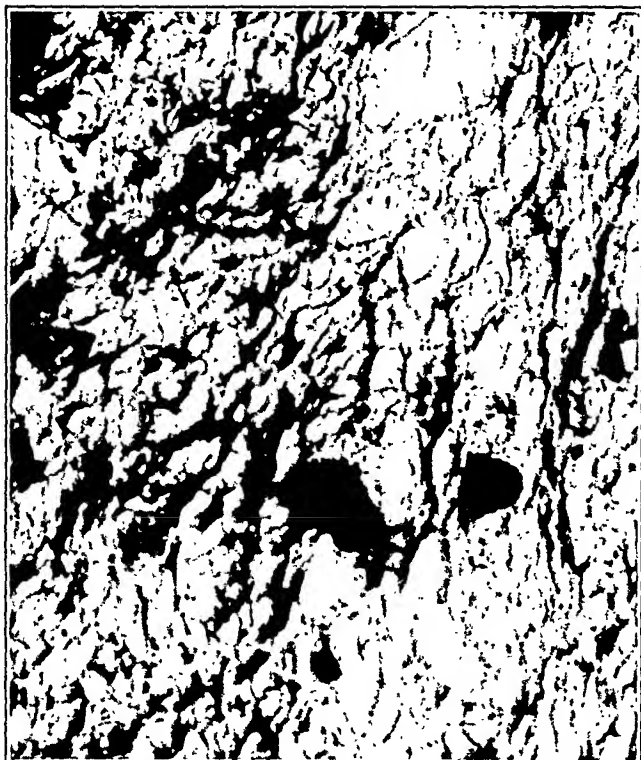
FIG. 1. Astrocyte from fibrillary astrocytoma. Phosphotungstic acid hematoxylin. Stain carried out upon formalin-fixed material after treatment by ammonia and refixation in Zenker's solution. $\times 1000$.

FIG. 2. Neuroglia fibrillae from a brain showing cortical sclerosis. Ethyl violet-orange G. Formalin-fixed material; ammonia treatment; Zenker refixation. $\times 1000$.

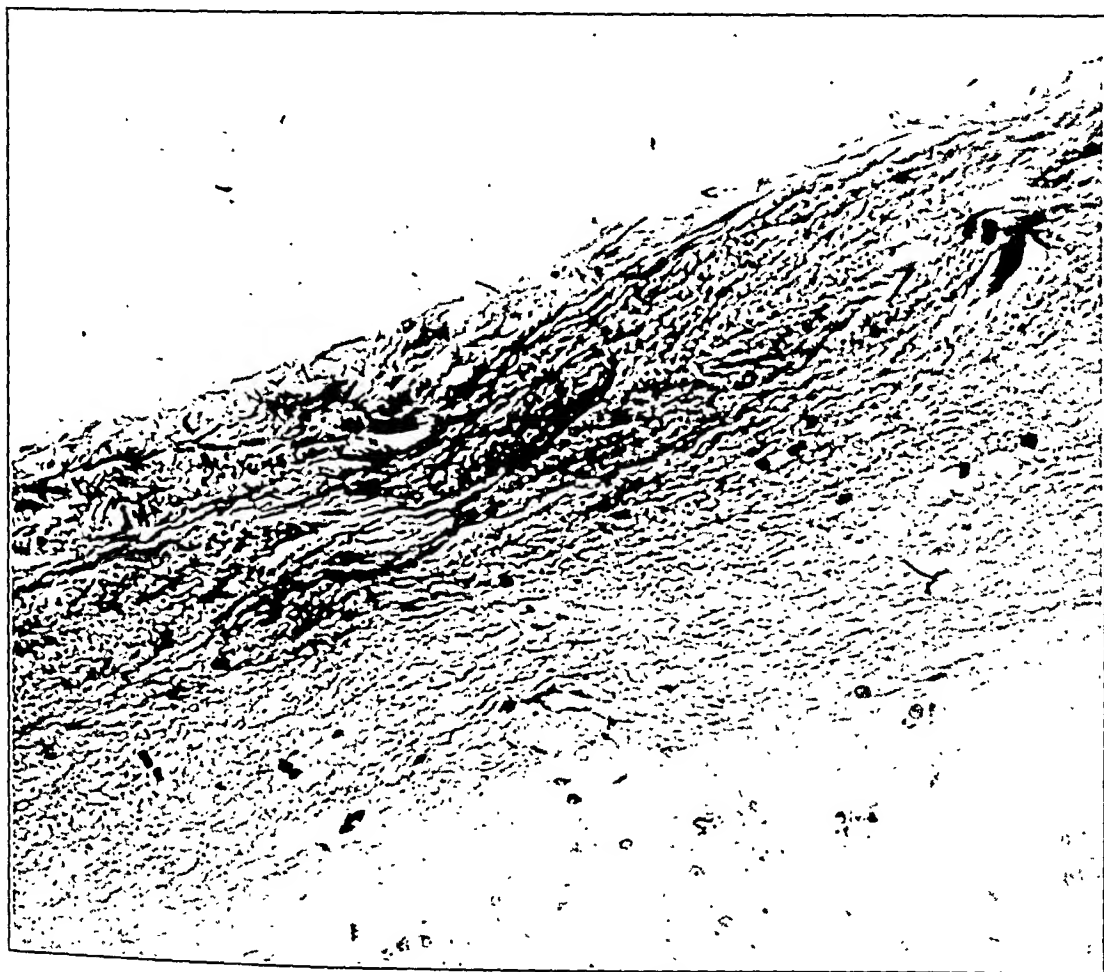
FIG. 3. Neuroglia fibrillae from the same case as Fig. 2. Ethyl violet-orange G. Treated in the same manner as the above. $\times 250$.



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2



3

MULTIPLE PRIMARY NEOPLASMS IN LOWER ANIMALS *

REPORT OF A CASE

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To the pathologist interested in the study of human tissue the occurrence of two or more primary heterogeneous neoplasms in the same person is not an uncommon observation. On the other hand, to the student of comparative pathology who is privileged to see a relatively small number of tumors in animals the finding of multiple primary neoplasms in an animal is likely to be considered a pathologic event, at least worthy of a brief description. I do not mean to infer that the lower animals are less prone to neoplastic proliferation than man or that in them there are fewer multiple tumors. The fact is that if the lower animals were permitted to live out their natural span of life most of them might show a tumor incidence comparable to that of man.

The apparent discrepancy in the incidence of neoplasms in man and lower animals is due to several factors, the most important of which are: (1) the comparatively early age at which the majority of animals, particularly the meat-producing animals, die, and (2) the failure to make a thorough necropsy examination on animals that die from natural causes. Even many veterinarians, whose training should have incited at least ordinary interest in morbid pathology, will not perform a necropsy unless especially urged to do so and necropsies by lay owners are not of any value in the acquisition of pathologic data. As a result only a small percentage of animals dying from natural causes are carefully examined by someone directly interested in the pathologic anatomy of disease.

The finding of more than one primary tumor in the same individual has always resulted in renewed interest in the subject; thus, many cases have been recorded in the literature. This is particularly

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true in cases occurring in human beings. Although foreign literature contains reports of many cases of multiple neoplasms in lower animals the cases reported in English literature are rare.

In this brief review an attempt has not been made to present a complete summary of the literature. Enough cases have been reviewed, however, to enable one to grasp a more comprehensive conception of multiple primary neoplasms as they occur in animals than would be possible from the one case reported herein.

Multiple tumors of the thyroid gland in a dog were described by Schöne.¹ In the thyroid gland was a typical primary carcinoma with extensive metastasis to the lung, and a spindle-cell sarcoma which failed to metastasize. Schöne believed that the sarcoma resulted from degenerating epithelial elements, or was at least secondary, and related to the carcinoma.

Betke² reported multiple primary neoplasms in a forty-year-old captive rhinoceros. In the uterus was a fibromyoma and in the mucosa of the cervix were two large papillary adenocarcinomas. Metastasis, even to the regional lymph nodes, was not demonstrated.

Wooldridge³ noted adenoma of the prostate and adenocarcinoma of the liver in one case. The animal was a fourteen-year-old fox terrier. The prostate contained a mass weighing 1 kg. In spite of the huge size of this tumor, normal micturition was possible. Multiple adenocarcinomas of the liver were also found.

Ball⁴ found in a nine-year-old poodle an epithelioma in the pancreas and a sarcoma of the intestine situated close to the pylorus. Metastasis to the lungs, spleen, and kidney was also noted.

Mettam⁵ recorded the case of a seventeen-year-old pointer with a recurrent adenocarcinoma in the anal region, metastasis to the pleura of the right lung and spindle cell sarcoma in the mesentery near the ileocecal juncture. The intestine was not involved. On the surface of the spleen were three tumor-like nodules.

Bartlett⁶ described multiple tumors in two dogs. In one there was adenocarcinoma of the thyroid gland with metastasis to the lung, mediastinum and pineal region, bilateral mesotheliomas of the suprarenal glands, and cavernous hemangioma of the liver; in the other, adenocarcinoma of the thyroid gland with metastasis to the lungs, a chondrosarcoendothelioma of the mammary gland and multiple adenomas of the suprarenal capsule.

Boyd, Fitch, Grinnells and Billings⁷ observed multiple adenomas

of the pancreas and a cavernous hemangioma of the liver in a ten-year-old Holstein cow.

Fox⁸ in an extensive experience with captive wild mammals and fowls recorded only one case, that of a jaguar (*Felis onca*), in which multiple primary neoplasms were encountered. The tumors were as follows: fibro-adenoma of the uterus, fibro-adenoma of the bile ducts, and lymphangioma of the mesentery.

Houdemer and Bablet⁹ described the case of a dog in which lymphoblastoma of the inguinal lymph nodes and papillomas in the penis were found.

In a paper dealing with old age in relation to cell overgrowth, Goodpasture and Wislocki¹⁰ recorded the finding of a total of ninety tumors in an examination of fifteen old dogs. The tumors in the respective animals varied from three to eleven. There might be some hesitation in accepting the entire ninety tumors as true neoplasms; nevertheless, the paper emphasizes in a forcible manner the tendency of tissues (at least in dogs) to advancing proliferation with the advent of senescence. In a later paper Goodpasture¹¹ discussed exhaustively the relation of the age of dogs to tumors and presented a summary of his observations in another series of thirty-five old dogs. In the fifty dogs studied 228 tumors were found, thirteen of which were classed as malignant and 215 as benign.

Perhaps the most comprehensive paper in recent literature dealing with multiple neoplasms in the lower animals is that of Cohrs¹² who, besides presenting an extensive bibliography, recorded his own valuable observations. In a total of 737 necropsies on dogs he found tumors in seventy; in twenty-six of these the tumors were multiple primary neoplasms. He described one case in particular in which there was a most unusual collection of neoplastic and related anomalies. The animal was a male dachshund aged 14 years. Clinical examination failed to reveal anything of diagnostic significance aside from polyuria and excessive thirst and the dog remained in good flesh until death. At necropsy a veritable museum of abnormalities was found: melanoma of the skin with beginning spindle cell sarcoma, multiple hard papillomas of the skin, ulcerated squamous cell carcinoma of the skin low in the median line, multiple sebaceous adenomas of the skin, multiple adenomas of the anal gland, papillary adenoma of the lungs, cortical malignant hypernephroma of the left suprarenal gland, cortical malignant hypernephroma of the right

suprarenal gland, primary carcinoma simplex and adenoma of the right testis, multiple carcinoma simplex of the left testis, nodular hyperplasia of the pancreas, and areas of nodular hyperplasia in the spleen.

A review of the papers of Goodpasture and Cohrs makes me somewhat hesitant to present the one case it was my privilege to study. I offer it, however, as a matter of record, not with the assumption that the observation of one case is important, so far as the conception of multiple neoplasms is concerned.

REPORT OF CASE

Clinical History: The dog was a thirteen-year-old male shepherd. From the time he was a puppy he had been fondly regarded as a member of the household and for a considerable portion of his life his habits had been sedentary. About two years before he died squamous cell carcinoma, graded 3, developed from the mucosa of the gum of the mandible in the region of the incisor teeth. This was removed surgically and healing ensued. About a year later recurrence was noted. The tumor continued to grow and to encroach on the soft tissues. The incisors were overgrown by cancerous tissue and the canines were loosened and pushed out of their normal position (Fig. 1). The lips were not affected. Debility increased gradually and for humane reasons the owner asked to have him killed.

Necropsy: Anomalies were as follows: a smooth, flattened, irregular tumor on the anterior floor of the mouth, the tumor encroached on the lower incisor teeth, which were loosened as were the canines; enlarged left submaxillary lymph node; enlarged thyroid gland, weighing 35 gm.; a large cyst 6 cm. in diameter in the liver near the periphery with many smaller pale areas just under the capsule, from 0.2 to 1 cm. in diameter, the surface of these areas being slightly depressed; large multiple nodules over the surface of the spleen; a grayish white irregularly spherical tumor measuring 2 cm. in diameter firmly adherent to the wall of cecum; prostate gland greatly enlarged, weighing 160 gm., and a yellowish mass irregularly spherical and measuring 1.8 cm. in diameter in the right testis (Fig. 2).

Pathologic Anatomy: Suitable material was selected from the various lesions and prepared for microscopic study.

The tumor of the mouth was a typical squamous cell carcinoma, graded 3 (Fig. 3), and similar in every respect to the primary tumor which had been removed two years previously. The tumor was covered by a thin layer of mucosa and near the surface were a few

areas of necrosis with infection. The trend of the growth was downward into the surrounding tissues rather than outward.

The left submaxillary lymph node possessed but few landmarks by which it could be identified as a lymph node. Large areas had undergone necrosis with dissolution of tissue, and the lymphoid cells were limited to a few isolated clumps near the periphery at one side. The bulk of the structure consisted of carcinomatous cells and a considerable amount of fibrous connective tissue. Many of the carcinoma cells were much altered in appearance and showed a decided tendency to assume an elongated rather than an oval contour. The influence exerted by the advancing fibrous tissue was apparent, although in spite of this inhibition the carcinoma cells retained a certain amount of aggressiveness and mitosis was easily demonstrated. Although the tumor cells show some alteration due to the associated connective tissue elements there can be no reasonable doubt that the tumor of the lymph node represents metastasis from the primary tumor in the mouth. There were many melanotic granules in what remained of the lymph tissue.

Sections of the liver were obtained through the pale depressed areas and these were found to consist of irregularly branching cavernous channels lined with a flattened type of cell which was apparently endothelial in nature (Fig. 4). The interior of many of the channels was occupied by variable quantities of red blood cells. The hepatic tissue in the tumorous areas was entirely obliterated. In some areas the channels were pushing into the adjacent substance which was in a state of congestion in the zone immediately surrounding the tumorous area. There seems to be sufficient evidence to consider this anomaly, hemangioma of multicentric origin.

The tumor of the cecum was a highly cellular, rapidly growing type of neoplasm which occupied a position in the wall of the intestine, extending from the serosa to the muscularis mucosae. In the tumorous area no vestige of intestinal musculature remained and the tumor was clearly encroaching on the adjacent muscle tissue. Even the muscularis mucosae was invaded in some instances by the advancing tumor cells although the mucosa was not disturbed. The cell was typical of smooth muscle tumor; the anomaly was designated malignant leiomyoma (Fig. 5).

Sections of the spleen were obtained from several of the splenic nodules. While the gross lesions were striking, the microscopic

picture was somewhat disappointing and decidedly difficult to interpret with any degree of confidence. The nodules consisted of extensive areas of red blood cells with a variable number of large irregular lymphoid cells, some of which appeared decidedly embryonic. The condition was considered multiple nodular lymphomatous hyperplasia.

The thyroid gland had suffered profound alteration and few elements remained which were common to the normal thyroid gland. Most of the alveolar spaces were filled with red blood corpuscles, and a meager quantity of colloid substance was present in a few of the remaining alveoli. The most striking change, however, was the tremendous increase in the interstitial connective tissue of the gland. This was so profuse in some instances as to cause a fusion of large areas with resultant hyalinization. As a consequence many of the alveoli were obliterated entirely. The diagnosis was chronic fibrous thyroiditis.

Normal prostate tissue was not found in any of the sections of the prostate gland examined. The structure was that of multiple cystic cavities lined with high columnar epithelial cells, the nucleus of which was situated near the attached end. While the epithelium lined many of the cysts in a smooth and orderly fashion, the reverse was true in the majority of the fields in which the epithelium was projected into the interior of the cysts from all sides in great profusion (Fig. 6). The rugae or papillae were of variable height and the high columnar type of epithelium was maintained throughout. A colorless, lace-like residue was present in several of the cysts. Mitosis was not observed in any of the cells. The amount of fibrous tissue was not increased and the epithelial cells did not show a tendency to invade the surrounding stroma. A diagnosis was made of papillary cystadenoma.

The tumor of the testis was sharply demarcated from the surrounding testicular elements by a zone of fibrous connective tissue and while the adjacent seminiferous tubules were clearly undergoing a retrogressive change they were in no immediate danger of being replaced by the neoplastic cells. The tumor cells were rather large, polyhedral, and closely packed together. Delicate strands of fibrous tissue separated small groups in rather indistinct units (Fig. 7). The cellular cytoplasm was inclined to stain lightly although the nuclei stained well. A nucleolus was observed in most of the cells, and

most of them had a rather high lipid content. Mitosis although present was observed infrequently. A diagnosis was made of interstitial cytoma of the testis.

Briefly summarized, the following morbid changes were observed: squamous cell carcinoma of the mouth with metastasis to the submaxillary lymph node; multiple hemangiomas of the liver; malignant leiomyoma of the cecum; papillary cystadenoma of the prostate and tumor of the interstitial cells of the testis; multiple lymphomatous nodules of the spleen, and fibrous thyroiditis.

COMMENT

The anomalies reported in this case represent five true tumors with splenic changes, perhaps a related neoplastic expression.

Considering the relative frequency of tumors of the thyroid gland in the dog it is somewhat surprising that the thyroid gland in this case should reveal chronic inflammatory fibrosis rather than a neoplastic tendency. Certainly the general condition of the animal was favorable although a certain amount of local inhibition must have existed, or all the tissues would have presented neoplastic changes. If the animal had been permitted to live until life was terminated as a consequence of the influence of the neoplasms or as a result of other senile delinquencies, it is probable that other tumors would have become demonstrable.

In considering the influences underlying the appearance of several primary tumors in the same individual, age seems to have much significance. This has been commented on many times by other observers. Goodpasture and Wislocki who based their opinion of age in dogs they studied by the presence of the usual senile changes, such as loss of teeth, the presence of cataract and the general decrepit condition, said: "In no animal with extensive wasting and loss of teeth have we failed to find tumors in more than one organ." Goodpasture remarked that the fifty dogs constituting the basis of his study were chosen because of evidence of advancing age and in no instance was a dog presented for study because of the presence of a tumor. Smith¹³ studied the senile changes of the testis and prostate in dogs; he used thirty-two animals varying in age from six weeks to twenty years. The fifteen animals that presented evidence of being ten years old or more than ten showed definite tumors at

necropsy. As regards the possible relationship of advanced age with the appearance of neoplasms in dogs, the observations of Cohrs are significant. Of the twenty-six animals in his study who had two or more primary tumors all but one were more than ten years of age.

The part that age plays in the occurrence of tumors, especially in dogs, is further emphasized by the relative infrequency with which neoplasms are seen in young animals. In a series of several hundred necropsies conducted during the last year very few tumors were found in dogs less than five years; most of them were found in the older animals.

It would seem that the explanation of the greater frequency of both single and multiple tumors in the older animals is definitely related to and dependent on senile involution of the respective tissues. Goodpasture said: "With age there is a progressive differentiation which eventually injures the cells of the body. Many of the cells die, others become dedifferentiated in varying degrees. The dedifferentiated cells possess the power to grow; but their capacity to function may be diminished or lost. From these dedifferentiated cells metaplasia and benign and malignant tumors arise."

The above hypothesis suggests the possibility that every tissue of the body may give rise to neoplastic overgrowth and ignores, or perhaps underestimates, the possible influence of heredity in the occurrence of tumors in the aged. It would be extremely important to know whether a dog with an established cancer-resistant ancestry which would protect it during the first ten years of life would develop single or multiple tumors due to a predominating influence exerted by the forces underlying the changes of senile involution.

The frequent occurrence of neoplasms in the older dogs makes it difficult to accredit heredity as providing the dog with any considerable amount of protection from tumors during the period of senile retrogression. On the other hand, it is difficult to believe that most dogs of advanced age possess a dormant hereditary susceptibility for neoplasms. Certainly there is a problem in genetics presented here which makes it impossible to know definitely what part, if any, heredity plays in the inception of lawless overgrowths of tissue in old dogs. Goodpasture's hypothesis that cells which have suffered injury due to age and have in consequence experienced loss of specificity and assumed an increased power to grow, appears insufficient to explain the entire phenomenon.

In the case of dogs neither sex nor breed appears to bear any relation to the incidence of multiple neoplasms.

SUMMARY

From a brief review of the literature it is apparent that primary, multiple neoplasms may appear in a great variety of species. Multiple neoplasms are frequently noted in necropsies on dogs more than ten years of age. This suggests the influence of senile retrogression and subsequent tissue involution in the etiology of these tumors. The possible influence of heredity is a factor which warrants investigation. A case of multiple primary neoplasms in a thirteen-year-old dog is presented. The tumors described are: recurrent squamous cell carcinoma of the mouth with metastasis to the regional lymph node; multiple hemangiomas of the liver; malignant leiomyoma of the cecum; papillary cystadenoma of the prostate, and a testicular tumor arising from the interstitial or Leydig cells. Multiple lymphomatous nodules of the spleen are also present.

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DESCRIPTION OF PLATES

PLATE 112

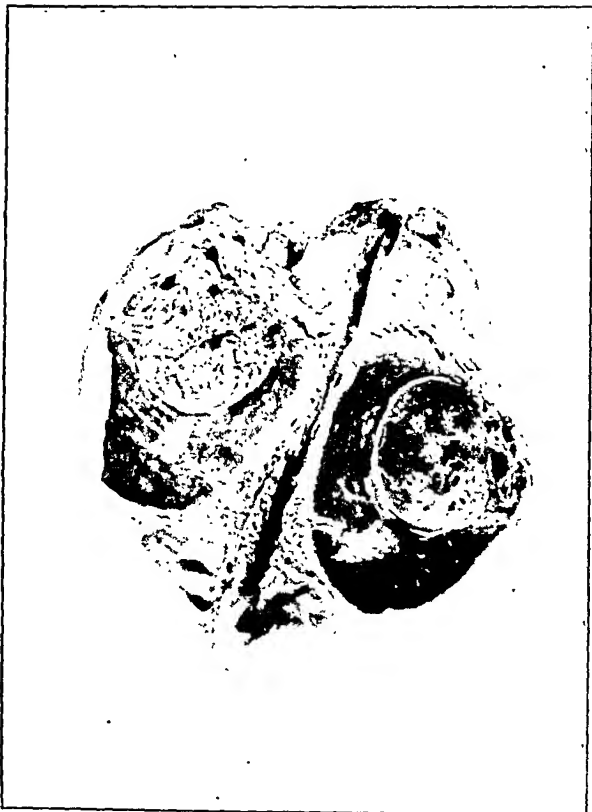
FIG. 1. Primary carcinoma of the anterior floor of the mouth. The cancerous tissue has caused the teeth to be pushed from their normal position.

FIG. 2. Interstitial cytoma of testes. Tumor is sharply demarcated.

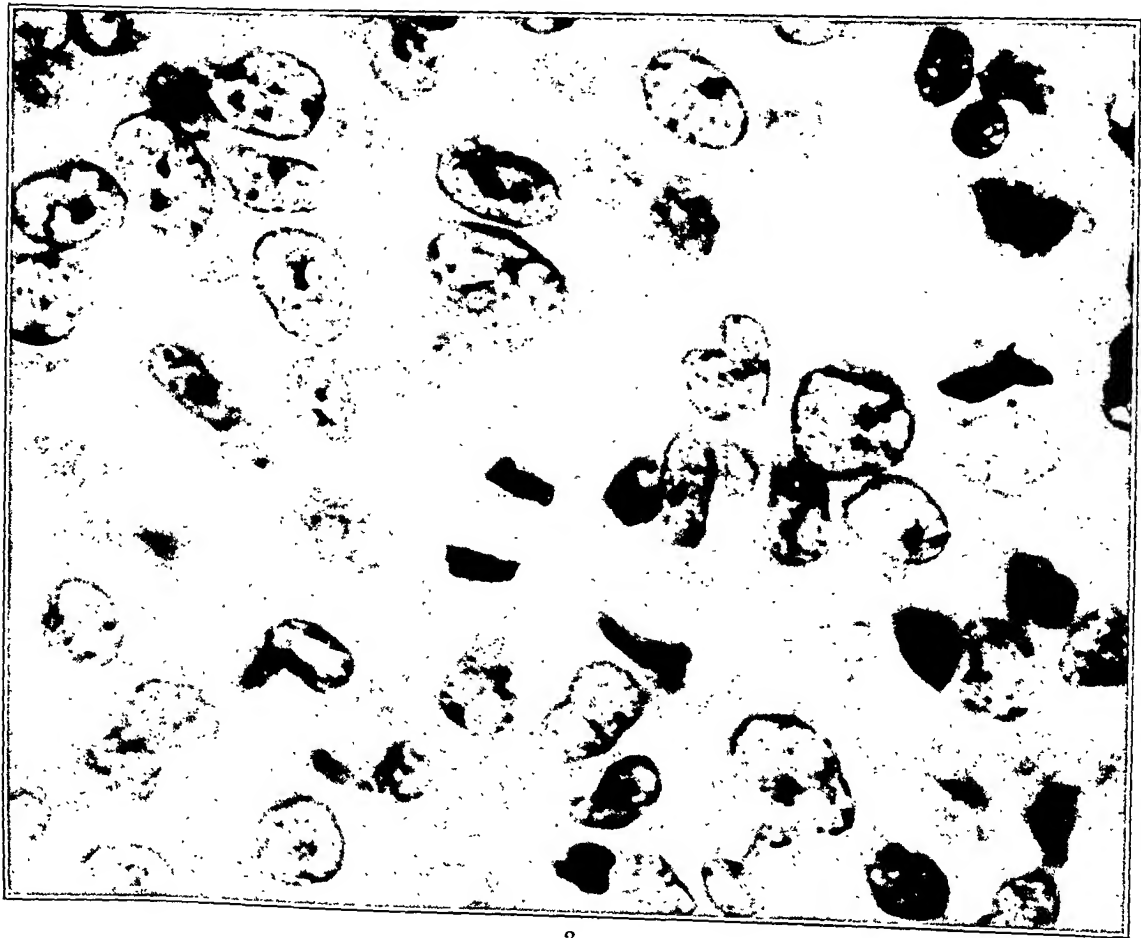
FIG. 3. Primary squamous cell carcinoma of the mouth. One cell in mitosis.
× 1350.



1



2



3

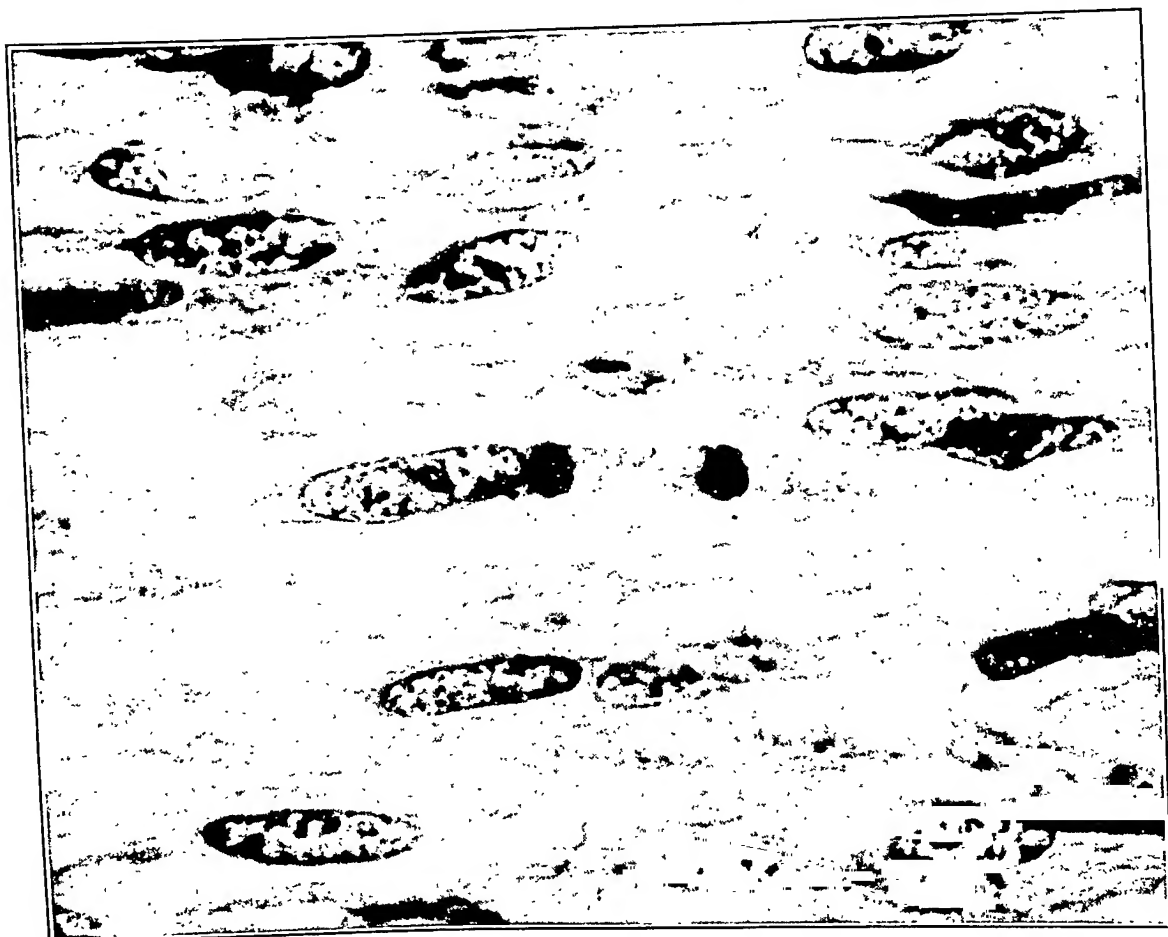
PLATE 113

FIG. 4. Hemangioma of the liver. Tortuous channels occupied by variable quantities of erythrocytes. $\times 50$.

FIG. 5. Leiomyosarcoma of the cecum. One mitotic figure present. $\times 1350$.



4



5

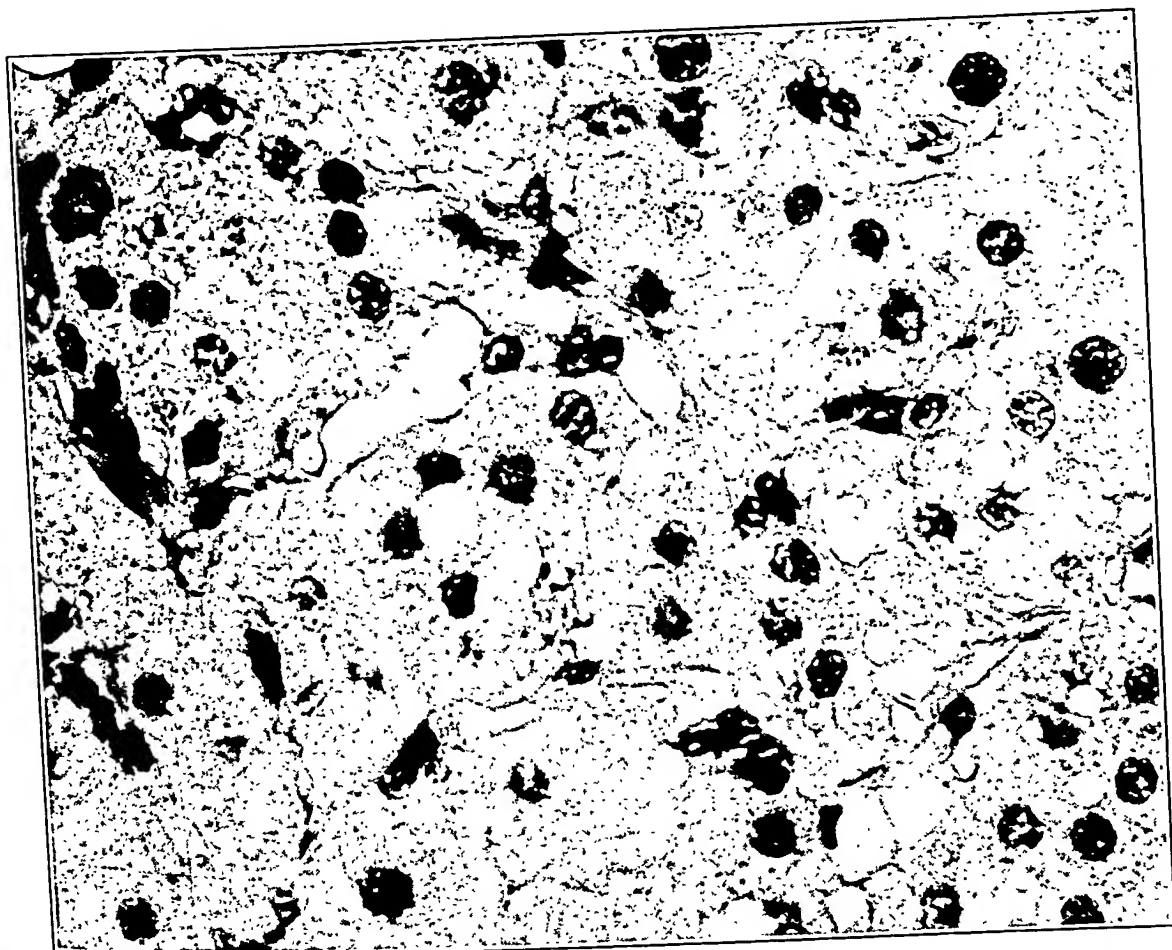
PLATE 114

FIG. 6. Papillary cystadenoma of the prostate gland. A richly cellular area showing the extensive overgrowths of cells, the majority of which assume a papillary type of arrangement. $\times 150$.

FIG. 7. Interstitial cytoma of the testis. Compact arrangement of the neoplastic cells; clear, somewhat granular cytoplasm and prominent nuclei. $\times 770$.



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PRIMARY MALIGNANT HEMANGIOMA OF THE SPLEEN WITH MULTIPLE LIVER METASTASES *

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Malignant tumors of the blood vascular system which metastasize to other parts of the body via the blood stream, are very rare. Less than a dozen such proved cases have been reported. Benign new-growths, on the other hand, are not uncommon and the literature abounds with descriptions of them and discussions of their origin and growth. These non-malignant neoplasms of the blood vessels may be localized and definitely circumscribed, or they may progress slowly, exhibiting a considerable amount of local proliferation and invasion. By Ewing¹ they are classed as simple hemangiomata (hemangioma simplex), of which he recognizes several types. The most important, in his opinion, are (1) the common, localized, non-progressive nevus vinosus of the skin, usually congenital in origin; (2) the subcutaneous plexiform angioma of young children, in which there may be considerable local invasion of the underlying structures; and (3) the more hyperplastic type of growth called by Ziegler the hemangioma hypertrophicum, in which the tumor cells grow quite rapidly and often show poor cellular differentiation.

These tumors may be further characterized as being either capillary or cavernous in type, according as the vessels of the growth have minute, capillary-like lumens, or appear as large distended spaces separated from each other by walls which are quite thin. Occasionally in these newgrowths vessel lumens are entirely absent,

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as in certain of the so-called endotheliomata, and the tumor cells grow in whorls or solid masses. By and large, however, benign angiomas consist of single or multiple circumscribed nodules in which small or large new-formed vascular channels are present.

The commoner forms of such angiomas appear on the skin or in the underlying subcutaneous layers. The face, scalp, labia, scrotum, extremities, and axillae are the regions chiefly involved. But the deeper tissues and organs may be affected as well. The majority of the growths are circumscribed and encapsulated, although not infrequently some of them evidence a certain local proliferation by the invasion and infiltration of structures in the neighborhood of the original growth. Cases of such local malignancy are on record, Hildebrand,² Gascoyen,³ Cruveilhier.⁴ Often these locally invasive neoplasms have led to the death of the patient.

A malignant hemorrhagic newgrowth of the skin of elderly subjects, first described by Kaposi⁵ in 1872, is also characterized by the formation of new blood vessels. In their initial stages the lesions of this disease are inflammatory and not neoplastic. They exhibit a definite relation to certain predisposing factors such as vascular disturbances, sclerosis, trauma, alcoholism, etc. Later, however, they may become true tumors, the malignant cells infiltrating the surrounding tissues and occasionally metastasizing to the viscera. These tumors, according to Pick,⁶ consist of new, dilated lymph and blood vessels about which the stroma is infiltrated with chronic inflammatory cells. Endothelial proliferation is prominent and undifferentiated sarcomatous cells are present in the perivascular tissues. Such malignant nodules are generally hemorrhagic, often pigmented, and may break down and ulcerate.

The nature of this disease is obscure. Some consider it to be an infectious granuloma of unknown etiology which, in its later stages, takes on genuine neoplastic properties. Others, however, believe the condition to be a tumor from the start, namely, a metastasizing capillary angioma which tends to undergo sclerosis. Vasoformative cells are certainly present, but because of its association with a chronic inflammatory lesion, its definite relationship to predisposing causes, and its tendency to ulcerate, this malignant growth differs from the primary blood vessel tumors about to be described.

True vascular newgrowths which exhibit the peculiar characteristic of malignancy, that of giving rise to the formation of metastases

in other parts of the body, are rare. Several cases, however, are on record. One of the most striking of these is reported by Borrmann.⁷ His patient, a young woman of twenty-six, had, in the skin of the right breast, an angiomatous growth which recurred repeatedly after operative removal. Ultimately the tumor led to the death of the patient by the formation in the lungs of multiple metastatic nodules of the same type. In this case the primary tumor was structurally a simple angioma, histologically non-malignant. The secondary nodules, however, exhibited somewhat greater cellular activity, but nowhere did the neoplasm have the microscopic characteristics of a malignant growth.

Ewing observed a similar case in which a primary hemangiomatous tumor appeared in the left breast of a female patient. This was followed by other tumors of the same nature in the skin and mucous membranes. There was evidence also of pulmonary involvement.

Shennan⁸ described the remarkable case of a woman of twenty-three whose history for six years had been marked by mediastinal pressure, hemoptysis, enlargement of the spleen, hemorrhage into the pleural cavities, and the formation of vascular nevi in the skin. At necropsy there were found multiple cavernous hemangiomata which involved the entire spleen, the thymus, the mediastinal lymph nodes, and portions of the lungs, liver, and bone marrow. Histologically the tumors were not malignant in appearance. It was felt, however, that the neoplasm originated in the mediastinum and that the other growths were undoubtedly of metastatic origin. Locally these tumors exhibited a certain invasive tendency.

Homans⁹ published a short account of the case of a young woman of twenty-two who was very anemic and whose abdomen had been enlarged for over a year. On several occasions bloody fluid had been removed from the peritoneal cavity. Laparotomy revealed a large abdominal mass of which a part was excised. Histologically it was an angiomatous tumor. Later the spleen was removed. It was infiltrated with multiple angiomata. The tumor was considered to be primary in the spleen with metastases to the other situations.

Langhans¹⁰ presented the case of a thirty-year-old man in whose left upper abdomen there was a large pulsating tumor. The mass grew rapidly larger and the patient died about two months after the onset of symptoms. On postmortem examination the spleen was found to be enormously enlarged and adherent to the diaphragm.

On section only a narrow zone of relatively normal splenic tissue could be recognized at the periphery of the organ. Histologically lymph nodules were not visible; trabeculae were present unchanged; the pulp was greatly altered. The remainder of the organ consisted of a firm fibrous stroma in which there were numerous blood channels containing brownish clots. Microscopically this part of the spleen was identified as a cavernous hemangioma in which endothelial cells were seen to be proliferating rapidly.

In the greatly enlarged liver there were many similar blood-filled tumor nodules which almost entirely replaced the normal parenchyma. Histologically these were definite angiomatous tumors of the cavernous type. In Langhans' opinion the spleen was undoubtedly the organ affected primarily and the nodules of the liver were metastatic in nature.

Theile ¹¹ described four cases of angioma of the spleen which were collected out of about nineteen hundred necropsies done over a period of four and a half years in the laboratory of Professor Lubarsch. Three of these were non-malignant and are mentioned very briefly. The fourth, because of its malignant characteristics, is worth reviewing carefully.

The patient was a fifty-six-year-old man in whose abdomen a large tumor could be palpated. This proved to be a greatly enlarged spleen which was removed surgically. The patient died the day after operation from internal hemorrhage. At necropsy the important additional findings were multiple newgrowths of a metastatic nature in the liver, lungs and stomach.

Macroscopically the spleen was very large, weighing 2500 gm. The capsule was thickened and covered with blood clots. On section little of the normal tissue could be recognized. Numerous whitish tumor nodules were present on the dark reddish cut surfaces. In places many large blood-filled spaces were seen. Histologically there were two types of growth, one in which the tumor consisted definitely of small and large vessels, the other where the atypical cells took on a sarcomatous character and grew rapidly, without great differentiation.

The liver contained many tumor nodules which resembled the splenic lesions. Microscopically some of these consisted of thin-walled capillaries in a fibrous stroma; others were more cellular, resembling the sarcomatous foci in the spleen.

Metastatic nodules were found also in the lungs and in the stomach. The pulmonary lesions resembled those in the liver, but the nodules in the stomach wall were sarcomatous in structure. No pure angiomas were found.

Theile considers these two types of growth to be but phases in the development of a single tumor of angioblastic origin. The differences observed histologically he believes to be anaplastic in nature. The tumor, therefore, he designates a "sarcomatous angioma" of the spleen which gave rise to multiple metastases.

Finally Jores¹² described a somewhat similar case of sarcomatous angioma of the spleen in which the organ was found to be much increased in size, about 3600 gm., and almost entirely necrotic. The liver was likewise enlarged, weighing 8600 gm. Both organs were infiltrated with angiomatous tumors which in some places were histologically benign, in others of sarcomatous nature. The primary tumor Jores considers to have been in the spleen, the liver involvement being secondary.

In these seven reports the angiomatous tumors which are described exhibit a common characteristic, namely, the formation of metastatic nodules of similar histologic structure in distant parts of the body. In four cases the tumors were histologically benign, but in those of Langhans, Theile, and Jores the newgrowths were definitely of malignant nature. Such tumors of blood vascular origin, however, are rare.

REPORT OF CASE

There came recently to the Boston City Hospital a patient whose history and clinical course presented a difficult diagnostic problem. At necropsy a large, blood-filled tumor of the spleen was found. Numerous neoplastic nodules were scattered throughout the splenic pulp and the enlarged liver was the seat of great numbers of secondary growths. Histologically the tumor proved to be a rapidly growing hemangioma of the spleen with multiple liver metastases. Because of its unusual features the case is here reported in detail:

History and Clinical Course: J. R., a white American laborer 25 years of age, was admitted to the Boston City Hospital December 12, 1924, complaining of pain in the back and swelling of the abdomen. His past history was unimportant. The present illness began about four weeks before admission when the patient received a sharp blow in the abdomen from a small hand truck which he

was pushing. For several days thereafter he had attacks of rather marked abdominal pain. The pain was sharp and knife-like, was situated in the loins and lower back, and radiated upward to the shoulders. Ten days before admission his abdomen began to swell. His skin was transiently yellow and at these times the stools were pale and the urine high-colored. The patient became progressively weaker, his appetite failed, and he lost about four pounds in weight.

The patient seemed to be in considerable distress, complaining constantly of pain in the lower back. The skin was pale but not icteric. The chest appeared symmetrical, with moderate fullness at the junction of lower chest and abdomen. The abdomen was distended. Over the epigastrium the percussion note was flat and slight tenderness was elicited on palpation. The liver edge could not be felt. Ascites was not noted.

At the time of admission his blood pressure was systolic 140 and diastolic 100; temperature 98.4° F; pulse 72; respiration 28. The red cell count was 5,280,000 per cmm. Leukocytes 14,300. Hemoglobin 75 per cent (Sahli). A blood smear stained with Wright's stain showed normal cells and a normal differential count.

The urinary examination was negative except for the presence of bile in moderate amounts. The stools were clay-colored. On the third day after admission the skin and sclerae were slightly icteric. Although the liver could not be definitely palpated it was felt that the fullness in the upper abdomen was due to enlargement of that organ. At this time ascites was found to be present.

Paracentesis resulted in the removal of 2150 cc. of slightly cloudy, bile-stained fluid which was transudative in character and sterile on culture. After the tap the mass in the upper abdomen was recognized definitely as a greatly enlarged liver. The spleen could not be felt. The removal of the fluid, however, brought no relief and the patient became more restless and the pain more severe. Two days later the abdomen was again full of fluid. The temperature gradually fell until it was subnormal. The pulse became more rapid and weak. The red count dropped to 2,728,000 per cmm., with hemoglobin of 60 per cent (Sahli).

A surgical consultant advised immediate laparotomy. After a preliminary transfusion of 600 cc. of citrated blood the abdomen was opened and the peritoneal cavity was found to be full of fresh and partly clotted blood. No ruptured viscus could be discovered. The spleen and liver were greatly enlarged and the surface of the latter organ was studded with soft, dark reddish nodules of varying size. The kidneys were apparently negative. A diagnosis of carcinoma of spleen and liver was made and the incision closed. In spite of active supportive treatment the patient's temperature remained subnormal and he sank gradually into unconsciousness, dying about sixty hours after the operation.

NECROPSY PROTOCOL

The body is that of a well developed adult white male. Nothing of note is found on superficial examination.

Peritoneal Cavity: The peritoneal cavity contains approximately 1000 cc. of dark reddish fluid in which there is a considerable amount of clotted blood. The serous surfaces, both parietal and visceral, are

inflamed and are covered with fresh fibrin, which mats together the coils of small intestine and binds the omentum closely to the surgical incision in the peritoneum. The liver is greatly enlarged and extends down to a point 11 cm. below the ensiform process and 8 cm. below the costal margin in the right mammary line. Its surface is seen to be studded with numerous soft, bluish red nodules which will be described more at length later. On pulling the stomach and the splenic flexure of the colon toward the midline, a large amount of clotted blood, much of which is old and friable, is found beneath these structures. Removal of these clots discloses the greatly enlarged spleen, to which they are adherent. This organ is situated normally but it is approximately four or five times its usual size. In many places its thickened capsule is firmly attached by fibrous tissue to the diaphragm and portions of neighboring organs. With difficulty these adhesions are divided and the organ removed.

Spleen: Weight 520 gm. The organ measures 15 by 5 by 8 cm. in size exclusive of a large, somewhat spherical tumor mass which is firmly attached to its diaphragmatic and gastric surfaces. This mass measures 10 by 9 by 9 cm. and its pedicle-like base at the point of origin in the spleen is approximately 7 cm. in diameter. It is quite definitely a large primary tumor of the spleen itself. The surface of the organ, exclusive of the tumor mass, is bluish red in color and is thrown into small ridges which give it a corrugated appearance. Here and there stringy fragments of soft clotted blood are adherent to the thickened capsule, and in many places there project above the capsular surfaces small, rounded, dark reddish nodules which are quite soft and collapsible. The shape of the organ, however, except for the projecting tumor mass, is well preserved.

The large spherical tumor is covered with old, granular blood clots beneath which is the thick, tough, yellowish capsule. In one place where the clotted blood is especially dense, there is an irregular capsular tear which measures 1.5 cm. in length. This opening is definitely of ante mortem origin, for its edges are covered with fibrin which is undergoing organization. On sectioning the mass the knife passes through the tough fibrous capsule and plunges into a series of large cavities which are filled with soft, dark reddish, necrotic, bloody material. In the center of the tumor and extending down into its pedicle, there is a core of yellowish white, firm tissue which measures 1.5 cm. in diameter. Imbedded in this tissue there is a

blood vessel filled with a long, friable thrombus which in many places is adherent to the vessel wall. The entire lumen of the vessel, however, is not occluded.

On section of the spleen the tumor mass is found to be completely encapsulated so that it is separated from the splenic tissue. It now appears to be but one of many tumors of this organ. The others are smaller and less distinct, and show little or no necrosis. They are scattered throughout the spleen appearing often immediately beneath the capsule, above which some of them project. The majority of the nodules, however, are present in the deeper tissues. In general these lesions are irregular, somewhat spherical cavities which vary considerably in size, the smallest visible ones measuring about 0.1 cm. in diameter, the largest approximately 1 cm. The latter are trabeculated, for thin, yellowish white strands of connective tissue are seen projecting into them or dividing them into smaller spaces. The most noteworthy characteristic of all of the nodules is that they contain considerable quantities of blood which gives them a deep reddish brown color and thus sharply demarcates them from the surrounding tissues. It is impossible to recognize the normal architecture of the spleen and between the individual lesions there exists a stroma of reddish, moist tissue which in places is markedly sclerosed. The nature of these hemorrhagic nodules is not at once evident. For the moment they are considered to be newgrowths of unknown type.

Liver: Weight 4250 gm. The organ is much enlarged. The capsular surface is moist and glistening but is studded with raised, rounded nodules which resemble the superficial tumors of the spleen. Most of these are soft and collapsible and appear dark bluish red in color. They vary greatly in size. Between them the liver capsule has a greenish brown hue with faint yellowish mottling. On sectioning the organ the entire parenchyma is found to be infiltrated with nodules similar to those seen on the surface. In shape these structures are irregularly spherical, varying in size from 0.5 cm. to 3 or 4 cm. in diameter. The largest lesions consist of trabeculated spaces filled with clotted blood. The smallest are yellowish and contain less blood. All of the nodules are clean-cut and distinct. The tissue of which they are composed is yellowish white and quite friable. Between these neoplastic growths, which are present everywhere in the liver, the parenchymal tissue is pale greenish brown in color and

appears markedly atrophic. A faint yellowish mottling, confined to the central portions of the lobules is noted.

Aside from slight congestion of the lungs and moderate scarring at their apices, the examination of the remaining organs shows no noteworthy changes.

MICROSCOPIC EXAMINATION

Spleen: In sections from the spleen the normal reticular tissue of the pulp is recognized only in occasional areas. Small, unaltered lymph nodules are rarely visible and most of the architecture of the organ is either destroyed or obliterated by an extensive neoplastic growth which invades and infiltrates the entire organ.

Microscopically this newgrowth is characterized by the formation of narrow channels and dilated cavities which are filled with blood. In some places these newly formed spaces are lined with atypical cells which grow rapidly and profusely, exhibiting the characteristics of malignant cells. In others the neoplastic nature of the growth is less evident and the numerous blood vessels are imbedded within a fibrous stroma which varies in amount and density in different regions. Those parts of the neoplasm which are least progressive resemble vascular scars. There is thus evident a wide variation in histologic structure. In all places, however, the tumor exhibits one common and outstanding feature, the formation of blood-containing channels.

The most characteristic portions of the neoplasm are those in which the tumor cells are growing rapidly and appear most atypical and undifferentiated. In such regions there are formed large abnormal spaces which are filled with blood. Most of these spaces are irregularly spherical in shape and measure from 0.5 to 2 cm. in diameter. Into them there project varying numbers of papillary processes on which the typical tumor cells grow profusely. These processes consist of thin, pink-staining strands of collagen in which no blood vessels or other structures are demonstrable, except rarely when the nucleus of a connective tissue cell or the lumen of a small capillary is seen. Such processes sometimes project inward from the wall to form a complex maze within the space (Fig. 2); sometimes they radiate from a vascular fibrous core which occupies a central position in the cavity. About this core the papillary structures are gathered in concentrically arranged strands.

It is readily seen that these projections are not simple processes of stroma which merely grow out as short, finger-like extensions into the adjacent space. They are long and delicate, and branch in many directions, forming an intricate maze of tortuous collagenous strands. Because of their profuse arborization they are cut in every possible plane so that they vary in shape from narrow strips to isolated circular or oval fragments which lie free in the blood-filled spaces. They thus resemble in many ways sections through placental villi.

Attached to the papillary projections in multicellular layers are the typical tumor cells. These are large, irregular in shape, and are so crowded together that they maintain their attachment to the underlying stroma by nothing more than delicate strands of cytoplasm (Fig. 7). They have thus an irregular, piled-up arrangement, in places resembling the structure of pseudo-stratified epithelium. The cytoplasm of these cells is scanty. Where it is seen it appears pale bluish in color, without specific structure. The nuclei are large and vesicular, often filling the entire cell. They contain small amounts of chromatin material in the form of minute granules scattered irregularly throughout a faintly reticulated nucleoplasm. Nucleoli are not present. Proliferation of these cells is rapid as is evidenced by the presence of numerous mitotic figures, as many as six or eight being found in a single oil-immersion field (Fig. 8).

Within the papillary-lined spaces, often closely surrounding the papillary structures themselves, are varying numbers of red blood cells. In some of the nodules they are present in great abundance, giving a deep red color to these lesions so that they stand out prominently even on naked-eye examination of the section. In other places, however, the blood cells are less numerous. Rarely, small fibrinous clots are found within the interpapillary spaces. Some of these are true thrombi, for there is injury and even necrosis of the adjoining tumor cells. Most of the clots, however, are of postmortem character.

Rapidly growing tumor nodules such as these just described are found throughout the spleen. Although they vary considerably in size, they exhibit everywhere the formation of dilated spaces which contain papillary projections in varying numbers and in different degrees of complexity. In a striking way some of the smaller lesions resemble a typical coccidial cyst of the liver in which projections of epithelium are characteristically formed. The blood spaces them-

selves may be seen to communicate directly with readily recognized blood vessels, and the atypical tumor cells which line them form a layer which is continuous with the normal endothelium of these vessels. Such abnormal spaces are seen, therefore, to be extensions of the normal vascular system.

The regions where tumor proliferation is least active are just as numerous as those where growth is rapid and invasive, but are less striking because of their resemblance to vascular scar tissue. In these situations sclerosis is marked and the blood vessels are small and well formed. The endothelial cells are less numerous, more highly differentiated, and only rarely exhibit active cell division. In general they resemble normal endothelium. In some places they are adjacent to or even surround rapidly growing nodules (Fig. 3). Foci of progressive infiltrative growth are thus often associated with regions of less marked neoplastic activity.

Between such extremes of tumor growth as have been described there are many other regions in which varying degrees of moderate neoplastic activity are evident. Here the atypical cells infiltrate the splenic tissue, adapting themselves to preëxisting stroma to form new blood vessels which are lined with large cuboidal cells. Such vessels are larger than the normal veins of the splenic pulp. They are usually numerous, being separated from each other by thin, fibrous walls. Not infrequently the tumor cells are found in the process of mitotic division.

The tumor, therefore, is of variegated histologic structure. In some places the growth is active, invasive, and malignant, and exhibits a tendency to form papillary projections within dilated spaces; in others there are diminishing degrees of endothelial proliferation, with a fibrous stroma which varies in amount and density inversely with the tumor activity. The neoplasm appears to have existed for a long time — months to years — for sclerosed, quiescent foci are present in most of the sections. But the rapidly growing, atypical tumor nodules are so abundant that one must consider the spleen to be now the seat of a highly malignant and progressive newgrowth in which blood vessel formation is the outstanding feature.

Sections from the large tumor at the upper pole of the spleen are disappointing. They show little of value histologically. The entire structure is infarcted and the tissues necrotic. Thick, degenerating bands of connective tissue divide the structure into smaller cavities

which contain large thrombi. Within the fibrous trabeculae there are numerous small blood vessels. In places there is evidence of papillary formation in some of the large spaces.

Liver: The lesions in the liver resemble those in the spleen. They are distributed throughout the organ but are most numerous in the neighborhood of the periportal spaces. Often the tumor completely surrounds these latter structures, infiltrating the adjacent parenchyma and causing atrophy and disappearance of the liver cells.

In the rapidly growing nodules the papillary processes are often more distinctly outlined than they are in the spleen and they present a less confusing picture. Here their tortuous, branching form is most clear and their arborizing character most evident (Fig. 2). In many of the nodules the processes originate from one portion of the wall of the space rather than from all parts equally.

Here and there in regions of less active growth there are large numbers of well formed blood vessels. In general these vessels are small, but not infrequently they reach the size of the channels which one finds in the common benign hemangioma (cavernoma) of the liver (Fig. 1). The endothelial cells which line these vessels are sometimes flat, sometimes cuboidal. They are more numerous, more primitive in appearance than are the endothelial cells which are found in normal blood vessels. It is possible to find a few mitotic figures in these cells so that they must be considered as actively proliferating, though not in the same profusion as those which are present in the more characteristic lesions where papillary processes are formed. In many places the tumor is sclerosed and resembles a vascular scar (Figs. 5 and 6).

The newgrowth everywhere infiltrates the liver tissue and atrophied parenchymal cells are readily demonstrated. Scattered throughout the connective tissue of the older lesions there are portions of bile ducts which stand out clearly from the fibrous stroma. Often the ducts are relatively increased in number, and in such areas, because of the absence of liver cells, the microscopic picture resembles in a striking manner that of acute yellow atrophy of the liver.

In several of these sections fragments of papillary processes carrying numbers of malignant cells are seen within preëxisting veins. In one such fragment there are two mitotic figures.

Careful histologic study of the remaining organs shows nothing of

note except for the presence of many endothelial leukocytes within the capillaries of the glomerular tufts.

The complete anatomical diagnoses are: Malignant hemangioma of the spleen with multiple metastases to the liver; infarct and rupture of large hemangioma of spleen; hemoperitoneum; acute fibrinous peritonitis; acute intracapillary glomerulonephritis, slight; healed pleuritis; healed tuberculosis of the lungs, apical; recent surgical incision of the abdomen; recent surgical incision of the antecubital fossa (site of transfusion).

DISCUSSION

The tumor which has just been described has three outstanding characteristics. First, it reproduces blood vessels and blood-containing spaces. Second, it grows rapidly and invasively. Third, it gives rise to the formation of secondary nodules in another part of the body. It is, therefore, a malignant, metastasizing hemangioma and falls into a group of very rare neoplasms of the blood vascular system. It originated apparently in the spleen, for in this organ the oldest and most sclerosed lesions are found. The liver became secondarily involved and at the time of death was extensively infiltrated with the growth. From a histologic standpoint this new-growth differs in many respects from the metastasizing hemangiomata which have already been reviewed.

The tumor described by Theile in some places exhibited the characteristics of a slowly growing hemangioma, while in others its growth was more malignant and invasive, the neoplastic cells forming cords or sheets without definite structure. From the foregoing description it will be noted that a somewhat similar condition exists in the present case. Neoplastic growth in some situations is rapid and malignant; in others it is diminished, and the tumor resembles a typical benign hemangioma. Where cell proliferation is most active, however, the endothelial cells grow not in undifferentiated groups or masses, such as Theile described, but as papillary projections which extend into dilated, blood-filled spaces, much as epithelial cells grow in a papillary adenocystoma of the ovary. The tumor cells are multiplying so rapidly that there is not available sufficient space for them to be laid down in a single layer. They thus grow in several layers, often buckling or projecting inward

from the surface and carrying with them small amounts of delicate fibrous stroma, to form the characteristic papillary projections. Thus the malignant cells here tend to line large, blood-filled spaces or to project into them so that they are always in contact with the blood stream. These cells, therefore, while they are still abnormal and grow in atypical fashion, are less primitive and undifferentiated than are those in the more malignant parts of Theile's tumor. In the more slowly growing regions the two neoplasms are similar.

In some ways the papillary processes of this tumor resemble the "Kolben" described by Borrmann. In his preparations, however, such projections were short and knob-like and the endothelial cells which covered them were flat and well differentiated, forming a thin, single-layered membrane. They were in no sense malignant. Between these "knobs" there were narrow, irregular blood spaces which were really out-pocketings from a larger vascular cavity. Such narrow spaces Borrmann called "blood vessels of the second order," and in his opinion actual circulation through them was very sluggish, a fact which he felt accounted for the presence of thrombi which he found not infrequently in these situations.

In the present case, on the other hand, the papillary structures are long and delicate and the spaces corresponding to Borrmann's blood vessels of the second order, are narrow and branching. Thrombi, however, are only rarely found. Most of the cells of the tumor thus obtain adequate nourishment by direct imbibition and they are generally healthy and active. In the few places where small thrombi are present, there is evidence of degeneration of the tumor cells in the immediate neighborhood of the thrombus. This probably indicates that the circulation through some of the abnormal spaces was not sufficient for complete nourishment of the tumor tissue. Thrombosis may thus be explained, as in Borrmann's case, on the basis of previous degeneration of the adjoining cavity wall due to inadequate blood supply. There is no evidence of ante mortem clotting from any other cause.

The type cell of the tumor is the endothelial cell, since blood vessel formation is the outstanding characteristic of the newgrowth. The blood circulates freely through the newly formed channels and comes into intimate contact with the neoplastic cells. In many places the tumor cells are poorly differentiated and little can be made out cytologically. But within many of the malignant nodules there is

a definite gradation from undifferentiated to differentiated cells so that one is led to conclude that the tumor is a primary angioma in which both abnormal and relatively normal endothelial cells may be found.

True angioblasts, from a histologic standpoint, are not found in sections from the tumor. According to Sabin,¹³ the characteristics of angioblasts are distinct enough to allow of their identification in sections. These cells, in the embryo, develop in solid or syncytial masses in which cell outlines can not be discerned. Later, by vacuolization or liquefaction of some of the cells in the syncytium, vesicles are formed, the walls of which become endothelium. There is a real differentiation of the cells thus left along the edges of the new formed vessels and the endothelial cell differs considerably from the angioblast, having a slightly longer nucleus and less granular cytoplasm. Careful study of the sections from the tumor here described fails to reveal any cells or cell groups which can be definitely identified as true "angioblasts." The neoplastic cells do not form syncytial masses. They tend to line spaces or cavities. True, they are not characteristic endothelial cells, but they appear to be less primitive than the embryonic angioblast. They are, in the writer's opinion, endothelial cells which have undergone neoplastic change and now grow wildly and profusely, penetrating and invading normal tissues or adapting themselves to the stroma of preformed blood vessels. They produce new, abnormal blood channels apparently by the method of sprouting, that is, by uncontrolled division and increase in the cytoplasm of the preëxisting tumor cells. This activity of the neoplastic cell thus resembles that of the mature endothelial cell which, as is well known, produces new cells by its own division, not by the addition of new, immature mesenchymal cells to the capillary wall. The evidence thus leads to the conclusion that the rapidly growing, atypical cells of this tumor originated not from primitive angioblasts, but rather from true endothelial cells. In their neoplastic state they reproduce endothelial structures.

Rapid proliferation of these neoplastic cells is evidenced by the presence of numerous mitotic figures (Fig. 8). Growth is most active in those regions where the papillary projections are conspicuous, for here the tumor cells are piled up on each other in irregular and atypical fashion. It is reasonable to assume that the rapid and uncontrolled growth of the cells in these regions, associated with

the occasional finding, in preëxisting vessels, of portions of papillae containing viable tumor cells, is indicative of malignancy and potential secondary tumor formation. The explanation for metastatic lesions is, then, not hard to discover. One wonders why the tumor is not more widespread. Perhaps such fragments of malignant tissue as were observed in some of the normal veins never reached the general circulation, being caught in narrow capillaries or sinusoids of the liver. Or it may be, as Handley¹⁴ maintains in the case of mammary carcinoma, that there are antagonistic influences exerted within the blood stream upon the tumor cells which gain access to it. But it would be astonishing should such antagonistic forces be operative in the case of endothelial cells, since in the blood stream these cells still remain bathed in their normal nutritive fluid. Our ignorance of the nature of the subtle changes which take place in tissues and fluids is still very great, and a discussion of the factors which either favor or prevent metastasis formation would be most speculative. Certainly there are metastatic tumor growths within the liver and none elsewhere. So much is fact. The forces which determine this distribution are unknown.

In the case under discussion there are not only regions of rapid growth such as those which have just been considered, but also regions of diminished activity in which the true character of the neoplasm is readily recognized. Here the fibrous stroma is increased in amount and the blood vessels, products of tumor cell activity, are numerous, resembling those found in the benign angiomas. Generally such vessels are capillary in type, although larger cavernous spaces are not infrequently found. Sometimes the endothelial cells in such foci exhibit certain atypical characteristics, such as a primitive, cuboidal form; a crowded arrangement simulating beginning papillary production; and the occasional presence of mitotic figures. They are thus malignant and slowly progressive. In general, however, the cells in the regions of diminished activity are well differentiated and line typical blood vessels. Such sclerosed angiomatous foci represent the oldest parts of the tumor where growth is now quiescent and sclerosis marked.

The large infarcted tumor of the spleen is thought to be of the same nature as the smaller lesions already described. Here for a long period of time there was evidently localized neoplastic growth with the formation of the circumscribed mass. The information

which can be gained from critical observation of sections from the tumor, leads one to believe that it does not differ essentially from the younger and more characteristic lesions already described. It is thus only one of many tumors, differing merely in age and size.

SUMMARY AND CONCLUSIONS

1. There is here described a case of primary malignant hemangioma of the spleen which metastasized to the liver, with the production of innumerable tumor masses in that organ.

2. The type cell is the endothelial cell and the tumor and its metastases are characterized by the formation of blood vessels and blood-filled spaces. The latter are of atypical and unusual appearance. They exist as small or large, cyst-like vascular cavities into which there project varying numbers of remarkable papillary processes which are covered with rapidly growing endothelial cells. These processes differ from any structures previously described.

3. Neoplastic growth in these foci is active, malignant, and invasive.

4. Alternating with the regions of rapid and atypical growth there are other foci in which typical blood vessel formation is more evident. Here neoplastic activity is diminished or quiescent and marked sclerosis is present. In these areas the growth assumes the form of fibrosed hemangiomata.

5. The tumor is remarkable for its rapid, invasive growth; its unusual histological structure; and the formation of multiple metastases.

6. A review of the literature concerning other cases of malignant, metastasizing hemangioma is given.

I wish to thank Dr. F. B. Mallory for his kind advice and assistance and for making the photomicrographs.

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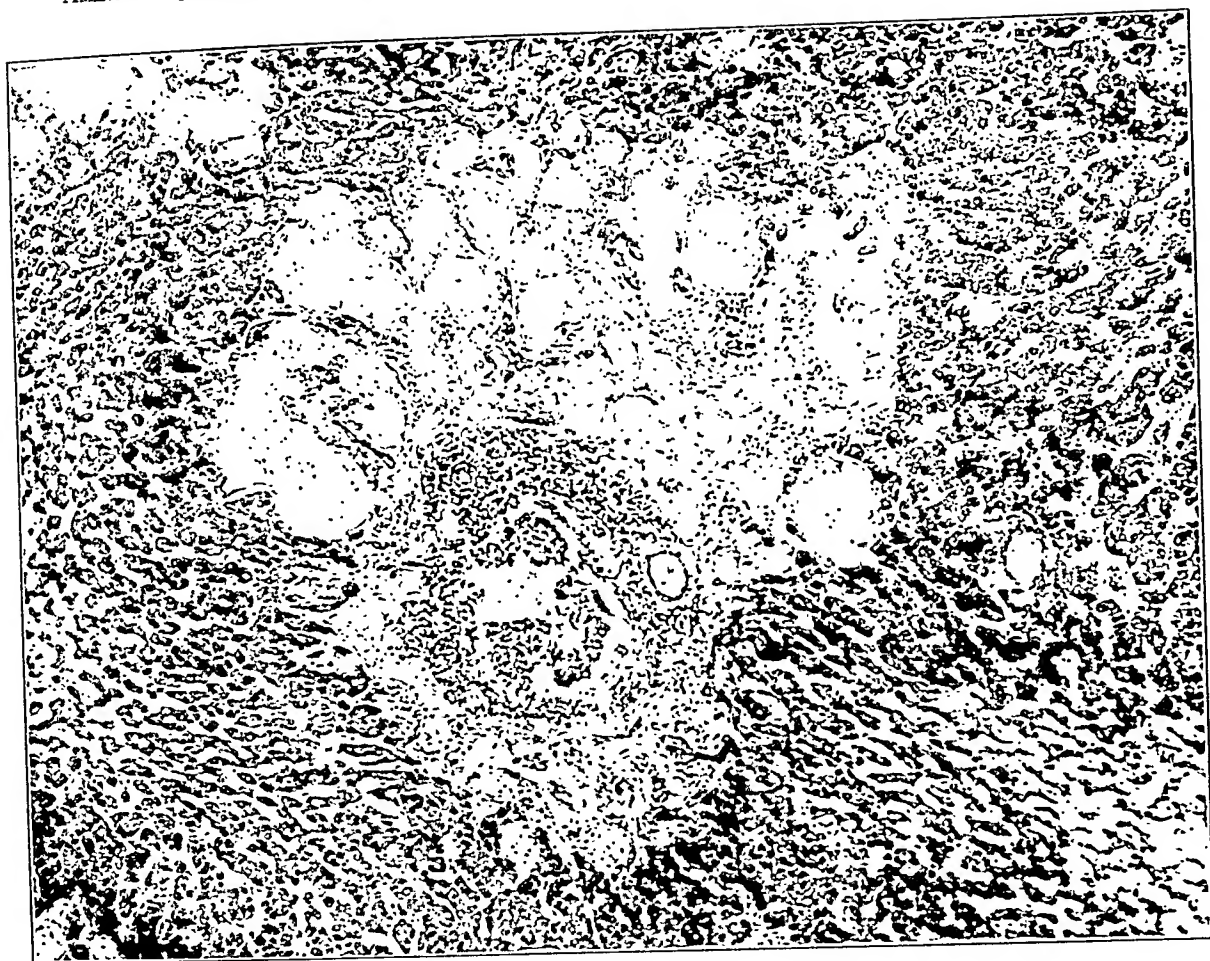
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DESCRIPTION OF PLATES

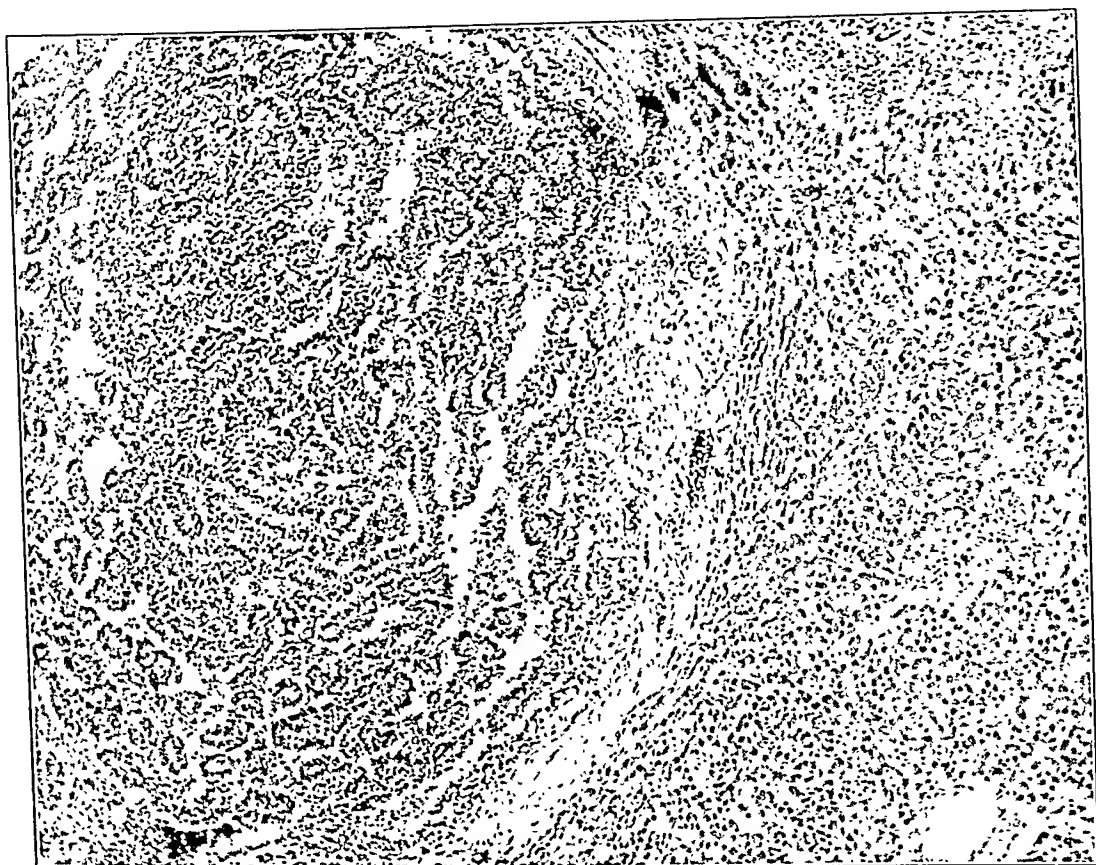
PLATE II 5

FIG. 1. Liver. A nodule showing three types of growth, cavernous, capillary, and papillary. Tumor cell proliferation is most active in the last-named region. $\times 80$.

FIG. 2. Liver. Large papillary nodule compressing surrounding parenchyma. Note the complex maze of papillary processes within the dilated space. Blood cells are present in moderate numbers between the projections. $\times 250$.



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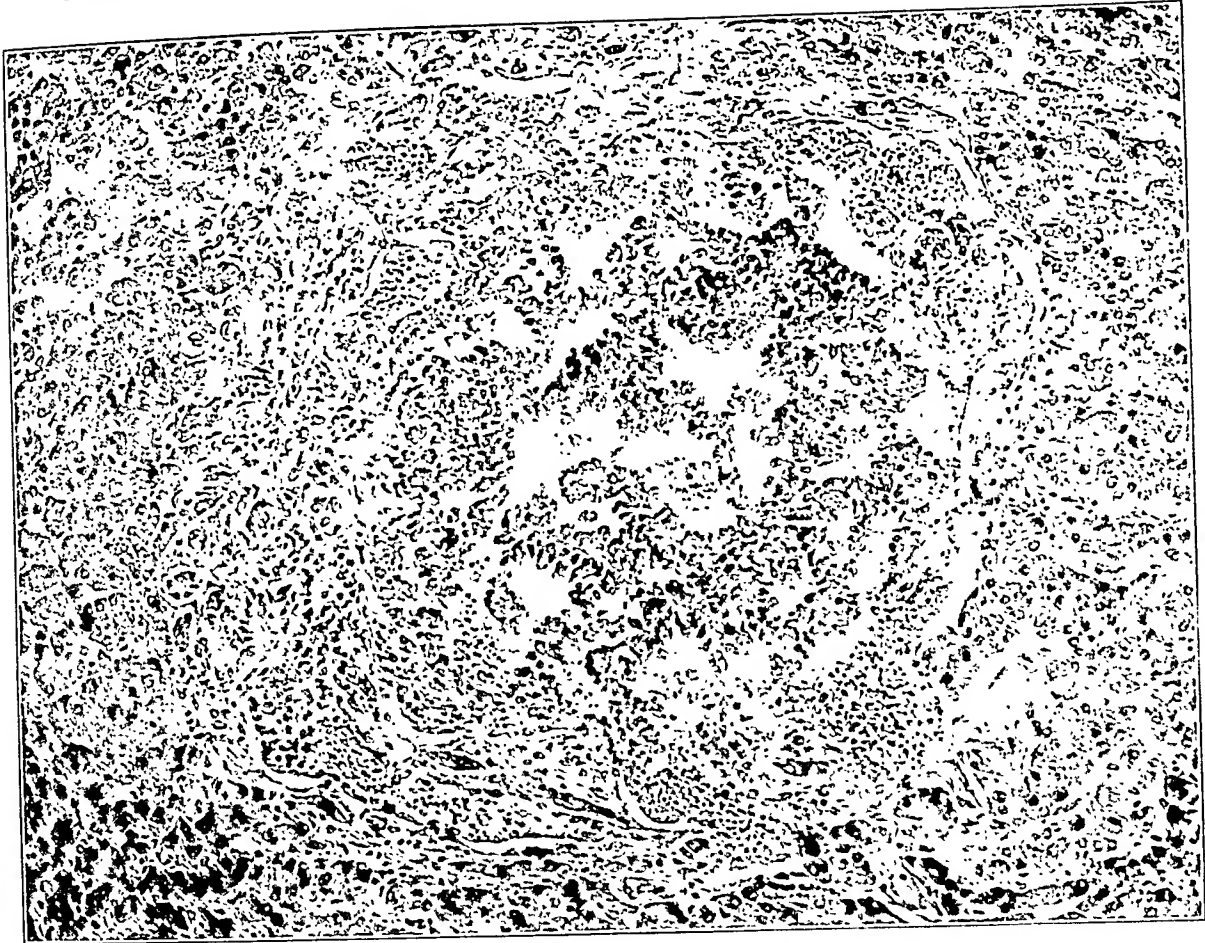
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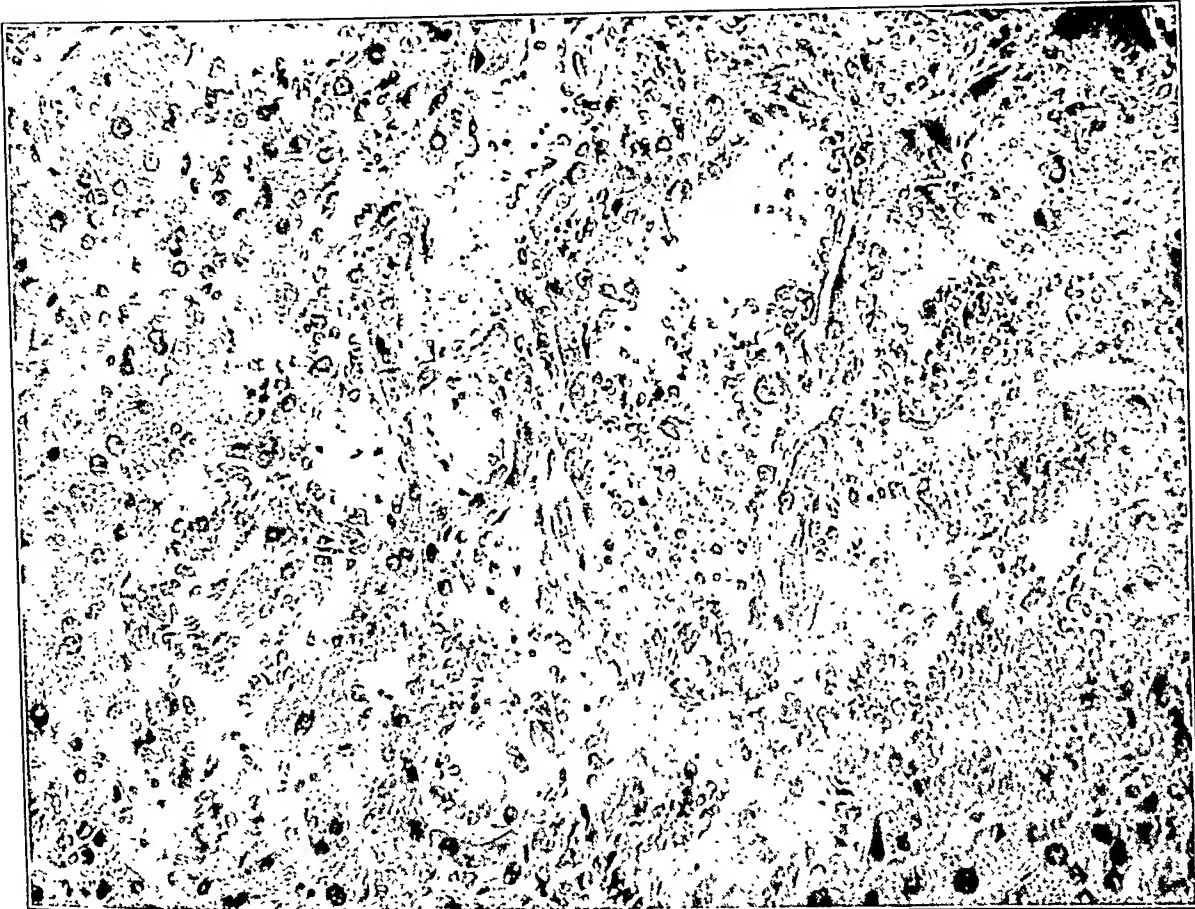
Primary Multiple Hemangioma of Spleen

PLATE 116

- FIG. 3. Liver. A nodule in which the peripheral tissue is sclerosed. In the center, however, is a mass of papillary projections on which the tumor cells are growing rapidly. $\times 150$.
- FIG. 4. Liver. An early lesion showing how the tumor cells adapt themselves to preëxisting vascular stroma. Sinusoids are dilated and lined with neoplastic endothelium. Liver cells are stretched and atrophic. There is no increase of stroma. $\times 250$.



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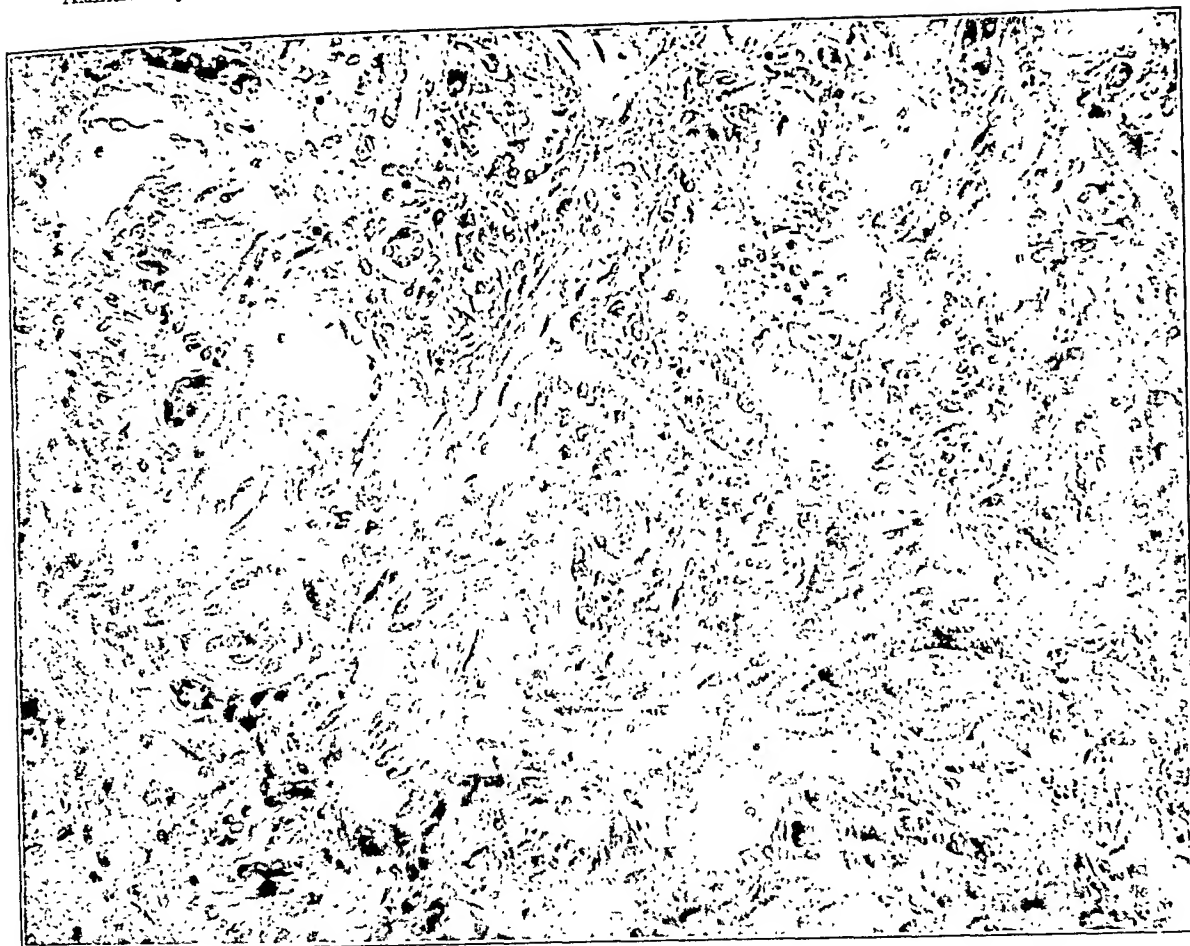


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PLATE 117

FIG. 5. Liver. A relatively late lesion. Many of the liver cells have disappeared. Those which remain are stretched and atrophic. Note the increase in stroma and relatively few vessels. The lesion is becoming scirrhou in character. $\times 250$.

FIG. 6. Liver. A late stage of scirrhou lesion in which many liver cells have disappeared. Note the increase and contraction of the fibrous stroma. The vessels are small and inconspicuous. $\times 200$.



5



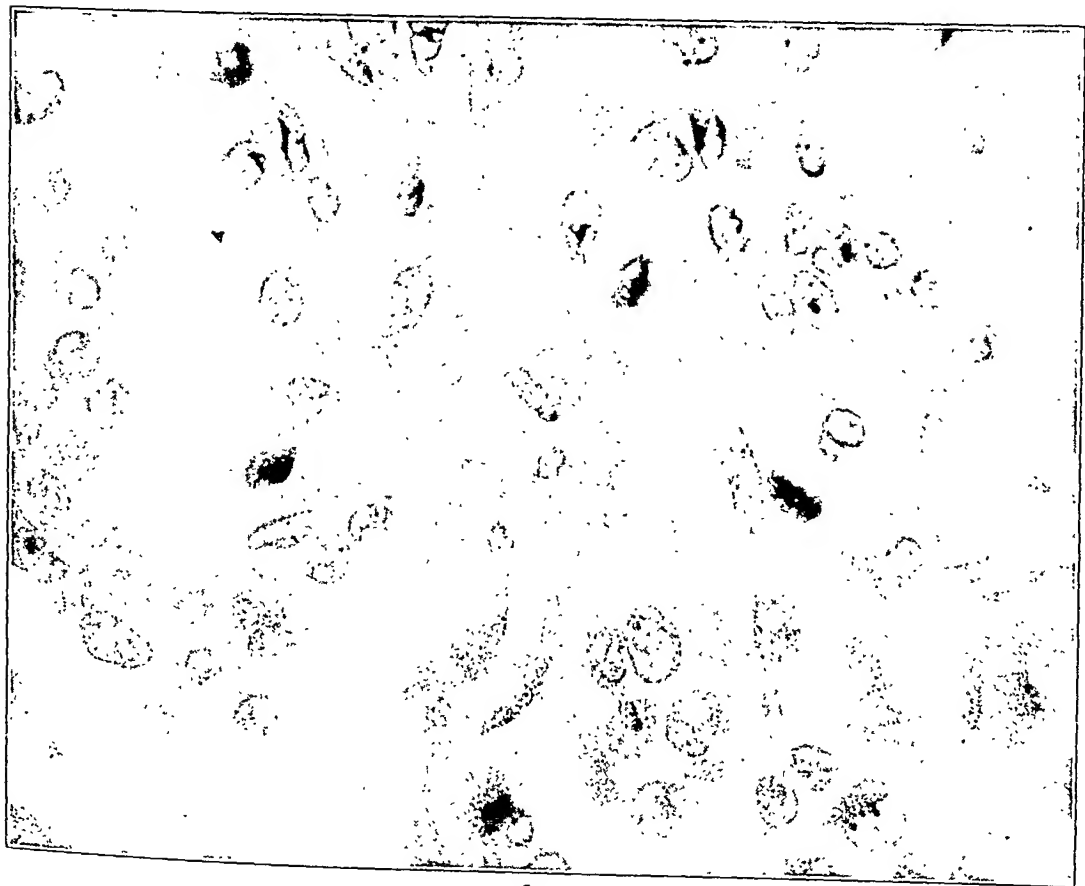
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PLATE 118

- FIG. 7. Liver. A higher magnification of the papillary processes to show their structure. These are non-vascular collagenous strands which are lined with the tumor cells. Note their piled-up, disorderly arrangement and the lack of cytoplasm. $\times 500$.
- FIG. 8. Spleen. A high magnification of one of the splenic lesions to show three mitotic figures which indicate rapidity of growth and hence malignancy. In this section the tumor appears as a capillary hemangioma. $\times 1000$.



7



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Primary Multiple Hemangioma of Spleen

CHEMICAL CONTRASTS BETWEEN COLLAGENOUS AND RETICULAR CONNECTIVE TISSUE*

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INTRODUCTION

It is now some thirty years since Mall¹ first described reticular tissue and differentiated it from the collagenous by means of tryptic digestion; it is almost twenty since a number of German investigators popularized the Bielschowsky-Maresch technic of impregnating reticulum with silver salts and studied its distribution. In spite of this, very little is known as to the chemical nature of collagen and reticulin. Shortly after Mall published his work, Siegfried² made a chemical analysis of the residue of pigs' intestinal submucosa after subjecting it to tryptic digestion and obtained the substance he named "reticulin." Mall had noted that reticular tissue did not yield gelatin on boiling with water and that it was more resistant to tryptic digestion than was collagen; he differentiated the two on the basis of these peculiarities and upon their morphological dissimilarity. Young³ contradicted Mall's contention, stating that the two substances were chemically identical as they both yielded gels after extraction with hot water. Siegfried, in turn, found that, although a substance resembling gelatin could be obtained by boiling reticulum in water, this was not true gelatin, but something more labile and more readily extracted; furthermore, there was a residue that could be precipitated with ammonium acetate or common salt, a grayish powder, his "reticulin." The gelatinoid material was obtainable after a short period of boiling, formed a firm gel, but differed from gelatin in that it yielded a heavy precipitate with acetic acid, formed no glutamic acid after boiling with hydrochloric acid for seventy-two hours, and contained phosphorus, which is absent in pure collagen and gelatin. Hammarsten⁴ gives the following composition for tendon collagen and reticulin, the latter being cited from Siegfried's paper:

* Received for publication June 9, 1928.

Reticulin: C, 52.88; H, 6.97; N, 15.63; S, 1.88; P, 0.34.

Collagen: C, 50.75; H, 6.47; N, 17.87; S, 0.57; P, none.

Siegfried's work was challenged in 1902 by Miss Tebb,⁵ working under Halliburton, and her paper elicited an answering article from Siegfried⁶ in the same journal and the same year. He had performed additional experiments to reinforce his statements and devised a more rapid method for isolating reticulin, by boiling the tissue in 0.05 per cent hydrochloric acid. His ideas have been accepted by Hammarsten and by Robertson⁷ and are quoted in their respective text-books. Abderhalden,⁸ on the other hand, contents himself with the mere statement that reticulin is probably an altered form of collagen, less readily convertible into gelatin, thus admitting a chemical difference between the two, but reversing Siegfried's opinion as to the ease with which reticulin is converted into gelatin.

It is difficult to find any definite statements on this subject, even in recently published text-books, although it is probable that much might be found in the voluminous literature on leather and glue research, could one but sift out the more fundamental articles from those of a technical or commercial type. Indeed, the subject seems to elicit very little curiosity in the minds of those who deal with these two connective tissue components.

In the realm of histology and histopathology, the question has been equally slighted; Strong and Elwyn's⁹ latest edition of Bailey's text-book and Prentiss and Arey's¹⁰ Text-Book of Embryology merely state that reticulum differs from collagen in its resistance to peptic digestion and in its staining reactions. The histological text-book mentions the possibility of reticulin's being a precursor of collagen. Miller,¹¹ in his study of the pulmonary reticulum, has said that this is a probability and, in subsequent publications and private conversation, has adhered to this belief. That reticulum is of a somewhat different chemical composition from collagen has been generally assumed by investigators of reticular tissue, but the very close relationship between collagen, reticulin and elastin has always been kept in mind.

Last year, Mallory and Parker¹² seriously questioned any chemical difference between collagen and reticulin; they consider that the silver method is incapable of differentiating two chemically unlike groups of fibers—it merely impregnates those that are readily accessible to the silver bath and fails to penetrate the denser, closely

felted and hence inaccessible bundles of collagen. They conclude: "Reticulum as a chemically distinct intercellular substance does not exist; it is collagen in separated form, rendered prominent by the silver stain." This statement, if generally accepted, closes the door on any further discussion of the subject; it is the purpose of this paper to prevent this by showing that there are chemical differences between reticulin and collagen and that they present a fertile field of investigation to the biochemist. So far as ascertainable, no one has attempted to link the biochemical with the microchemical method of attacking this problem, and it is proposed, in the following report, to do just that. The work will be divided into an histological and a biochemical section and the two will be correlated in the discussion; the spleen has been chosen as the organ of election for this undertaking, other organs being used for confirmatory and secondary objects of investigation.

HISTOLOGICAL SECTION

TECHNICAL METHODS

Methods of Fixation: 10 per cent neutral formalin (4 per cent formaldehyde); 10 per cent formalin with 0.5 per cent HCl and 0.5 per cent NaOH (to test out acid and alkaline fixation); Zenker's fluid; Bouin's fluid; absolute alcohol, acetone and ether (respectively); coagulation with boiling water and alcohol.

Sections: Paraffin embedding was used as a routine, although frozen sections were employed in part of the investigation and celloidin was used in the case of the Bouin-fixed tissue and frequently flooded over sections to prevent their becoming detached from the slides.

Impregnations and Stains: The Hortega silver-ammonium carbonate method was used as a routine impregnation, either alone or with a counterstain of Harris' hematoxylin and Van Gieson's stain.

Directions for Impregnating: Wash the deparaffinized sections in distilled water, after removing mercuric chloride with alcoholic iodine and hypo, in the usual manner. Chromium salts may be removed with KMnO_4 and COOH_2 (see later). After washing in distilled water, impregnate the sections for 15 minutes in a silver-ammonium carbonate bath which is made up as follows: to 10 cc. 10 per cent AgNO_3 , add 40 cc. 5 per cent Na_2CO_3 ; allow the white precipitate to settle, decant the supernatant fluid, wash with distilled water and

redécant, dissolving the precipitate by adding, drop by drop, just enough strong NH_4OH to put it almost into solution, leaving five or six gray granules undissolved; make up to 100 cc. with distilled water. Although the impregnation may be carried out in the cold, it is more satisfactory to warm the bath to the steaming point, driving off the excess NH_3 , and cool to 50°C . Then place in an incubator at 37°C , or thereabout, for 15 minutes.

Wash the sections in two or more changes of distilled water and reduce them with 1 per cent formalin, for two minutes. Wash at the tap and tone for two minutes in 1:500 aqueous AuCl , which tends to render the impregnation more precise and converts the yellowish brown collagen into grayish pink, or old rose. After washing, the sections are treated with 5 per cent hypo ($\text{Na}_2\text{S}_2\text{O}_3$) to remove excess silver or gold. The process depends upon the impregnation of the tissue by the double salt of silver and ammonia and its conversion, probably by reduction, into a black compound. That the double salt is silver-ammonium oxide, rather than silver-ammonium carbonate, is probable. It is usually advantageous to counterstain the sections for five minutes in Harris' hematoxylin followed by forty-five seconds in Van Gieson's picric acid-acid fuchsin solution, with a wash between. The sections should be run directly into ascending strengths of alcohol, otherwise water will decolorize the acid fuchsin. Van Gieson's solution is best made up of 10 per cent of 1 per cent aqueous acid fuchsin to 90 per cent of picric acid, water saturated at room temperature.

Staining Characteristics: The simple, untuned impregnation colors reticulum black, collagen yellowish brown; toned with gold chloride, the reticulum is still black, the collagen grayish pink to old rose; counterstained with Van Gieson, the reticulum is unaffected, while the collagen becomes bright vermilion. If the reticulum be unimpregnated with silver salts, it will stain rose-red with Van Gieson's solution.

Other Silver Methods: Another Hortega "variant," in which saturated Li_2CO_3 replaces the Na_2CO_3 ; the Bielschowsky-Maresch silver-ammonium oxide method, as modified by Mènard and myself;¹³ the Achucarro silver tannate method (Foot¹⁴) and some minor modifications of these were all employed as controls. No marked difference could be detected between these and the routine procedure, while many of them are more laborious and complicated. It may be said,

however, that the modified Bielschowsky-Maresch method gives the most reliable and clean-cut pictures, if one wish to devote the time to it.

HISTOLOGICAL EXPERIMENTS

Effects of Fixation: It was found that, of the fixatives mentioned, only one made any marked difference in the resulting impregnation: absolute alcohol prevented the blackening of the reticulum fibers almost completely; all the other fixatives gave good results and acted very similarly. In order to determine why absolute alcohol should inhibit the impregnation, a set of simple experiments, aimed at lipin extraction, were performed.

Unfixed Frozen Sections: Fresh spleen was cut on the freezing microtome and treated by the routine method; no impregnation of the reticulum or collagen resulted, although the refractile fibers were clearly visible.

Alcohol-Fixed Frozen Sections: Sections of fresh spleen were then soaked in absolute alcohol and ether for two hours, after which they were fixed to slides with celloidin and took the usual impregnation (Fig. 1). Where the sections were thin, the reticulum seemed broken up and the argyrophil material was diffused through the tissue; where they were thick, the impregnation was satisfactory. Next, sections were soaked in this mixture over night and treated as usual; the reticulum no longer became impregnated, there were a few black beads and clumps of reduced silver (Fig. 2). To test the fixation, sections were stained with hematoxylin-Van Gieson, with good success.

Paraffin Sections: Tissue fixed in ether, absolute alcohol, absolute acetone and 5 per cent NaOH was subsequently fixed in 10 per cent neutral formalin and embedded in paraffin. The sections from this material became impregnated very much in the usual way, excepting in the case of spleen treated with NaOH, in which the reticulum failed to become black; the collagen was unaffected. This rules out the possible extraction of lipins that might combine with the silver as a factor in the failure of alcohol-fixed tissue to become impregnated, for all these fluids are fat solvents. Some other explanation must, therefore, be sought.

Fixation in Boiling Water or Alcohol: Boiling water was used as one of the types of fixation to be tested, but it proved more interest-

ing as an extractor. Thin blocks of spleen were boiled for 4.5 hours under a reflux condenser in distilled water; half of them were then removed and run into paraffin, the remaining half being again boiled for the same length of time in absolute alcohol. To avoid injuring the knife on these bone-hard blocks, they were softened over night in 80 per cent alcohol three parts, to glycerol one part. This rendered them soft and they cut more easily than the water-boiled blocks.

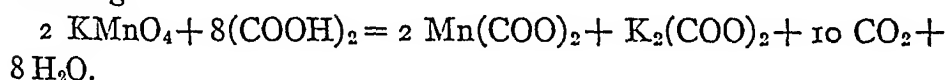
The following changes were noted in sections from these two lots of tissue after impregnation: in the water-boiled spleen the reticulum was clumped into swollen, somewhat dendritic masses of black material (Fig. 3), occupying the normal site of that tissue. The collagen was *quite unstained*, a counterstain with Van Gieson's solution brought out a faint roseate tinge in the capsule and trabeculae, but the bright vermilion was no longer forthcoming. The elastic tissue now appeared as isolated, sinuous strands; if the impregnation were preceded by treatment with the oxidizing mixture (KMnO_4) + $(\text{COOH})_2$ these took on a brilliant orange. Sections from the alcohol-boiled spleen were quite similar to those fixed in cold absolute alcohol.

EFFECT OF OXIDIZING AND REDUCING AGENTS UPON THE IMPREGNATION OF RETICULUM

I have noticed, in experimenting with silver impregnations of brain tissue, that the process was definitely affected by previous treatment with oxidizing and reducing agents. Mènard, in this laboratory, and the technicians at the U. S. Army Medical Museum at Washington (personal communication) have found that reticulum will impregnate better if first treated with permanganate of potash and oxalic acid. As this might depend upon (a) oxidation, (b) a salt of manganese, (c) oxalic acid, both oxidizing and reducing agents; a manganese salt and oxalic acid alone were applied to reticular tissue fixed in a variety of ways (see "*Fixation*") in order to ascertain what differences would result.

(A) *Oxidizing Agents*: KMnO_4 , 0.25 per cent, for five minutes, followed by oxalic acid $[(\text{COOH})_2]$ for ten, was first tested. The reticular impregnation was sharply intensified; fibers not demonstrable after simple formalin fixation (Fig. 4) became black and clear-cut after "oxidizing" (Fig. 5). These photomicrographs were from serial sections from the same block of tissue. The treated section

showed fibers that were somewhat stouter than those in the controls, the collagen was pinkish, if not counterstained with fuchsin, somewhat lighter than the controls if this were used; the cytoplasm and nuclei became paler and insignificant, which is an advantage — for they may then be better counterstained with organic dyes. The same feature was demonstrable with Zenker-fixed material; the untreated controls showed somewhat sharper impregnation of the reticulum than did the formalin-fixed controls, and the cytoplasm was brownish as a result of the formation of silver chromate; the “oxidized” sections showed sharper impregnation of the reticulum and markedly inhibited impregnation of the cytoplasm and nuclei. It is this fact that makes our (Foot,¹² Foot and Mènard¹³) modifications of the Hortege method peculiarly adapted for use with routine, Zenker-fixed material. In Bouin-fixed spleen, the results were materially the same, but after alcohol fixation, the impregnation of the reticulum was always slight, although the counterstain took very well. Subsequent fixation of alcohol-fixed tissue in 10 per cent formalin rendered the tissue suitable for first-rate silver impregnation. The reaction involved in the permanganate-oxalic acid treatment does not indicate oxidation on the face of it, as will be seen from the following:



Other Oxidizing Agents: Sections were treated respectively with commercial peroxide of hydrogen, acidified KClO_3 (1 per cent with 0.5 per cent HCl) and 30 per cent NaNO_2 with HCl to acidity. They were then impregnated as usual, always with untreated sections as controls in each lot. The reticulum was no better, if as well impregnated in the oxidized sections, although the nuclei were more sharply and precisely demonstrated. This would seemingly point away from oxidation as a factor in the improvement seen after the KMnO_4 — $(\text{COOH})_2$ treatment, but it might depend upon the more feeble oxidizing power of these reagents.

(B) *Potassium Permanganate Alone:* This was tried alone in 0.25 per cent concentration for five minutes. After impregnation, as compared with untreated controls, the manganated sections were much better, but rather spotty and filled with a brownish deposit from the manganese salts; the reticulum was, on the whole, more sharply impregnated. As the silver-bath contains NH_4OH , this

would oxidize the KMnO_4 as follows: $2 \text{KMnO}_4 + 2 \text{NH}_4\text{OH} = 2 \text{K} \cdot \text{NH}_4\text{MnO}_4 + \text{H}_2\text{O} + \text{Oxygen}$; continuing: $2 \text{K} \cdot \text{NH}_4\text{MnO}_4 + 2 \text{H}_2\text{O} = 2 \text{MnO}_2 + 2 \text{KOH} + 2 \text{NH}_4\text{OH} + 2 \text{Oxygens}$. This, then, may be construed as a true oxidation.

Potassium Permanganate and Hydrochloric Acid: In order to test out a more powerful oxidizing combination, KMnO_4 was used as usual and followed by ten minutes in from 1 per cent to 3 per cent HCl . The reaction here is: $2 \text{KMnO}_4 + 6 \text{HCl} = 2 \text{MnCl}_2 + 3 \text{H}_2\text{O} + 5 \text{Oxygens}$. The permanganate was not bleached out of the sections by the HCl as it was by the $(\text{COOH})_2$, and the resulting impregnations were very dense. The reticulum was probably even better blackened than in the oxalic acid technic, but the background was so intensely impregnated that the contrast was very slight and the reticulum obscured. Compare Figs. 6 and 7, made from the same block, the former with oxalic, the latter with hydrochloric, acid.

Inert Manganese Salt: It might be thought, in spite of this, that the superior results after KMnO_4 were due to the presence of the Mn ion, therefore sections were treated with $\text{KMnO}_4 + \text{HCl}$, as above, for a control and with MnCl_2 , followed by HCl . As compared with the controls, the MnCl_2 sections were vastly inferior, which would rule out the Mn ion as a controlling factor.

(C) *Oxalic Acid Alone:* As this is a constituent of the most successful method, it was used alone for 10 minutes in 5 per cent concentration. There was no impregnation of the reticulum, although it was well demonstrated in the controls. This should be compared with the results obtained with the reducing mixture, as given later on, for oxalic acid is a feeble reducer.

EFFECTS OF REDUCING AGENTS ON IMPREGNATION

A mixture of pyrogallol and NH_4Br , 0.5 per cent each and thiosulphate and sulphite of soda, 1 per cent each (in distilled water), was applied to sections for 15 minutes in warm solution before impregnating them with silver; this totally inhibited any impregnation of the reticulum (Fig. 8), except when absolute alcohol had been the fixative, when a partial impregnation (superior to untreated controls) resulted. The mixture of reducing agents was found to be superior to any one of them used separately. The function of the NH_4Br is not known; it possibly restrains the reduction, as in photography. While this is not striking proof, it at least strengthens the

assumption that the oxidation effected by KMnO_4 is the explanation of the improved impregnation following its use. (Cf. "*Oxalic Acid Alone.*") Alcohol-fixed tissue probably impregnates better after treatment with the reducing mixture for the same reason that it does after treatment with 10 per cent formalin, which is a reducer. The function of reduction and oxidation in this process must be further investigated; it is very bewildering.

EFFECT OF THE HYDROGEN ION CONCENTRATION OF THE FIXATIVE ON IMPREGNATION

As acids figure largely in the pretreatment of sections when KMnO_4 is used, it might be considered as the determining factor. Against this assumption is the unsuccessful impregnation after NaNO_2 and MnCl_2 , both of them followed by HCl . To strengthen this proof, sections were made from spleen fixed in 10 per cent formalin to which was added 0.5 per cent HCl in the one case, 0.5 per cent NaOH in the other. In both instances the sections became fairly well impregnated, but treatment with KMnO_4 and $(\text{COOH})_2$ produced the usual brilliant and superior impregnation; acidity or alkalinity of the fixative, therefore, appear to play no part in the process.

OTHER SILVER IMPREGNATIONS

The action of KMnO_4 and acid was found to improve the impregnation of sections by the Bielschowsky-Maresch technic, the Hortega Li_2CO_3 variant and the Achucarro silver tannate technic; the reducing mixture inhibited impregnation in all these methods.

REMARKS

All this indicates that it is best to treat sections with KMnO_4 before impregnating with silver. This should be followed by 5 per cent oxalic acid, if a light background suitable for counterstaining be desired; it should be followed by 1 per cent to 3 per cent HCl , if a denser impregnation is wanted and counterstaining is dispensed with. The great difficulty, in this method, is the liability of the sections becoming detached from the slides in the silver bath. This may be avoided (a) by careful drying of the mounted sections in the paraffin oven for forty-eight hours and a day or two at room temperature; (b) by driving off the excess NH_3 in the silver bath be-

fore impregnating, cooling to 50° C before the sections are immersed; (c) by removing the slides from the "descending absolute alcohol," after deparaffinizing in xylol, and flooding them with very thin celloidin (as in Mallory and Wright's frozen section technic), allowing this to set and continuing with the hydration, through 95 per cent alcohol.

BIOCHEMICAL SECTION

Preliminary Experiments: The first thing to do was to check up on Siegfried's original findings, therefore, splenic tissue was treated as he treated intestinal submucosa. Human spleen was used for two reasons — it contains more reticulum than most organs, has plenty of collagen for comparison and had been used in the experiments just recorded in the histological section. Spleens, fresh from the necropsy table, were ground in a meat mill, thoroughly washed in water, followed by several changes of distilled water and digested for two or three days with 0.25 per cent Parke, Davis & Co. pancreatin and 0.5 per cent NaHCO_3 , in distilled water at 37° C. The solution was filtered off each day through a kitchen strainer, renewed, and toluol added to keep down putrefaction. Finally the tissue was washed in 0.05 per cent HCl and boiled in the same concentration of the acid under a reflux condenser for two or three hours. The connective tissue broke up into a muddy precipitate, mostly without going into complete solution, although there was considerable reprecipitation on adding NaCl.

The precipitate and broken-down tissue were dried by gentle heat and some of the material was fixed in formalin, run into paraffin and sectioned; when stained, the sections showed some unmistakable tissue-débris, bits of capsule, trabeculae, consolidated reticulum and the like (Fig. 8). The remainder was extracted with chloroform and alcohol, but the extracts were so crude that more exact methods were used later. Thus far the results bore out Siegfried's statements in many particulars; there was far more tissue débris than he described and the watery extract would not jell. Siegfried did not examine his reticulin with the silver method, which had not yet come into being, so it was imperative to use it here. The bits of capsule and trabeculae were found to contain argyrophil fibrils and there were clumps of black material adhering to their margins, evidently compacted reticulum. This stained blue with Nile blue

sulfate, showing that it was not blood, which is faintly greenish yellow with that dye. Extracted "reticulin" showed somewhat less argyrophil material when impregnated with silver salts. There was a good deal of elastic tissue in the residue, which was to be expected, as it is very resistant to tryptic digestion and weak acids.

Following this experiment, a dozen or more were run off, each one representing increased refinement in technic and giving somewhat different results. Over ten kilograms of spleen were thus treated. It was found best to slice the organ, rather than to grind it, cutting slices about 2 mm. thick. It was also found necessary to wash the material repeatedly after digestion, in distilled water, in order to get rid of the last traces of digested cellular material. This was best accomplished by rotating the splenic framework in a liter of water in a beaker, using a glass stirring-rod belted to a low speed motor.

All the vicissitudes of these experiments need not be recorded here, but it would be well to indicate that a bewildering number of substances were isolated in earlier trials, subsequent improvement in technic showing many of them to be impurities. Siegfried's "reticulin" was recovered by boiling the digested splenic framework in dilute HCl, as already noted, the "collagen" fraction being separated by tedious filtration. It was found, however, that the following method, in which the "reticulin" was completely dissolved in NaOH and reprecipitated by neutralization and salting out, gave the best yield of the two fractions and simplified their separation.

ROUTINE METHOD FOR ISOLATING RETICULIN

About half a kgm. of the organ, at a time, was sliced, washed and digested in the incubator with either Parke, Davis & Co's., or Squibbs' pancreatin, 0.25 per cent to the liter of distilled water and 0.5 per cent sodium bicarbonate. After twenty-four hours' digestion at 37° C, the foul fluid was run off through a strainer, the material that was held back was washed in the mechanical separator for an hour or two in frequent changes of water, followed by distilled water, and then put back to digest in fresh fluid for another day. This usually sufficed; if not, another day's digestion was enough. The splenic framework, now reduced to an oyster-colored network of lacy reticulum and coarser trabeculae, was given a final

cleaning in several changes of tap water, followed by distilled water, in the washing machine, and it was then boiled in 200 cc. of distilled water under a reflux condenser for two or three hours. The tissue shrank considerably and gave off a yellow extract, *the aqueous extract*.

Following this, the extract was filtered off and set aside, the residue being rinsed in distilled water and digested in the incubator with 0.5 per cent NaOH for twelve or more hours. A peculiar greenish brown, fluorescent fluid, with scraps of undissolved connective tissue (elastin?) as a sediment, resulted. The residue was filtered out and again treated with NaOH, to insure as complete solution as possible, the dissolved material being pooled with that of the first run.

If the reader recall the histological section, the reason for these two steps will be clear; it was found that hot water extracted all the fuchsinophil material from the splenic trabeculae and capsule, while NaOH extracted the argyrophil material from the reticulum. What more natural, then, than to boil first in water to extract the "collagen" and follow this by digestion with NaOH to extract the "reticulin"?

The alkaline solution was then filtered through two thicknesses of filter paper in a Seitz vacuum filter, refiltered through four layers and then through an asbestos disc; the filtrate was deep brown, the process time-consuming. Hydrochloric acid was then added carefully to the filtrate until it was just acid to litmus paper. A putty colored precipitate immediately formed and was much increased by adding NaCl in considerable amounts, 5 per cent. The precipitate was then removed by centrifugation and dried over a water-bath, whereupon it became an amorphous, blackish powder. This was extracted for four hours in a Soxhlet apparatus, with 95 per cent alcohol, which removed a heavy extract of deep brown lipins. This was followed by extraction with distilled water for a like period, to remove the salt and any water-soluble material that might remain. Caprylic alcohol was added in small quantity, to keep down the troublesome foaming. The extracted residue was then dried over a water-bath, becoming a dark brown, somewhat crystalline powder.

DESCRIPTION OF FRACTIONS

Aqueous Extract: When evaporated to dryness over a water-bath, this yellow, water-soluble fraction became a glue-like sheet of brittle, hygroscopic material that powdered into flat, shining, brown flakes. On dissolving this in a little water, spreading on a microscopic slide, drying and fixing in Zenker's fluid, it could be handled like a section. After the routine silver impregnation and Van Gieson counterstain, it became bright red and showed no argyrophilic material; there were fibrillar artefacts, resulting from coagulation, that resembled collagen fibrils. With Van Gieson's stain alone, it stained rose-red; with acid fuchsin, somewhat deeper; with Mallory's connective tissue stain it became light blue; with Weigert's elastic tissue stain, pinkish; with silver carbonate alone, yellow-brown to amber. Commercial gelatin, treated in the same way, stained yellow with picric acid in Van Gieson's solution, orange with the orange G. in Mallory's stain, and deep brown with silver carbonate alone. Therefore our aqueous extract apparently differs from gelatin.

It gave a heavy, flocculent precipitate with tannic acid and a lighter one with Zenker's fluid; gold chloride, hydrochloroplatinic acid and other heavy metals produced varying degrees of turbidity. It did not reduce Fehling's solution and gave a pinkish biuret reaction. Its reactions, therefore, resembled those of pure gelatin to a certain extent.

Sodium Hydroxide-Soluble Fraction: Under the microscope, before alcoholic extraction, this appeared as a mixture of amorphous yellow granules, brownish plates and small crystals. Under the polarizing microscope, the crystals were anisotropic. Some of the yellowish granules proved to be iron-containing pigment, derived from splenic deposits and readily removed by treatment with HCl. The anisotropic crystals disappeared after alcoholic extraction. The partially purified precipitate, after neutralizing the alkaline solution, when fixed and impregnated on a slide, became impregnated with silver and did not take the acid fuchsin of the counterstain. It was composed of two sorts of material, black granules or even threads, and yellowish brown amorphous masses with some crystalline material, presumably salt (Fig. 9). The fibrils were sometimes continuous, sometimes beaded, and the impregnated and counterstained smear showed a striking resemblance, in its color scheme, to sections

impregnated by the Hortega method. Unimpregnated control smears showed no black material and the threads were invisible until impregnated with silver. These were not always obtained, sometimes the argyrophil matter was purely granular, particularly in the purified reticulin.

Alcohol Soluble Fraction: This extract was a deep mahogany-brown. On cooling, a precipitate settled out, identified as a mixture of glycolipins. It was very inconsiderable in bulk, reduced Fehling's solution and gave a modified Pettenkofer's test with sulphuric acid, without the addition of sugar. Dried on a water-bath, about one-third of the remaining material was found to be acetone soluble and to contain fats, fatty acids and some cholesterol; it gave a positive Salkowski reaction. The remaining two-thirds, soluble in cold alcohol and insoluble in acetone, was found to be composed of impure lecithin; it gave a Pettenkofer reaction with sugar and sulphuric acid, became whitish and swelled in water, and formed myelin figures when properly treated. These lipins gave very unsatisfactory impregnations with silver as they were difficult to hold on glass slides and the pictures were rather atypical. They do not seem to be the determining factor in the argyrophilia of the reticulum.

REMARKS

It may be said, then, that it is possible to extract a material with the staining properties of the collagen of histological parlance, by boiling the digested framework of the spleen in distilled water; it is also possible to dissolve the residue from this in weak caustic soda and to reprecipitate a substance from this solution by neutralizing with hydrochloric acid and salting-out with sodium chloride. Alcohol extraction removes lipins from this, leaving an insoluble residue which may be redissolved in sodium hydroxide, reprecipitated by acidifying and purified of the contained salts and water-soluble material by extraction with distilled water in a Soxhlet extractor. The purified product is very similar to Siegfried's description of his "reticulin."

This method was applied to spleen as a routine measure, but similar treatment of lung and heart, both rich in reticulum, gave precisely similar results, excepting that there was a copious, insoluble residue (resisting boiling in weak acids or alkalis) in the case of the lung, presumably largely composed of elastin.

In short, extraction with hot water has been found to remove the fuchsinophil material from connective tissue fibers and NaOH to remove the argyrophil. Hot water extract, on evaporation and fixation in Zenker's fluid, is strongly fuchsinophil; sodium hydroxid extract, when dried and purified of the lipins and water-soluble substances, is argyrophilic (after formalin fixation and treatment with KMnO_4 and $(\text{COOH})_2$) and does not stain with fuchsin. This would argue that there are two extractable substances in connective-tissue, leaving elastin out of the reckoning; the one may be extracted without destroying the fibers, the other (recovered after alkaline digestion) probably representing the digested fibers and the argyrophil material that coats, or is closely bound up with them.

DISCUSSION

What are "Reticulin" and "Collagen"? These terms must first be defined, a difficult task.

Reticulin: (a) From Mall's standpoint: a tissue that resists tryptic and peptic digestion and gives no gelatin on boiling with water.

(b) From Siegfried's: a tissue composed of two substances that resist tryptic digestion; one of them soluble in boiling, weak hydrochloric acid and gelling on cooling, the other precipitating out with ammonium acetate or sodium chlorid. Reticulin contains phosphorus.

(c) From the point of view of the histologist: any reticular connective tissue becoming black when impregnated with double salts of ammonium and silver.

(d) According to Mallory and Parker: merely a separated, more compactly fibrillary form of collagen fiber.

(e) From the viewpoint of many of us, frankly agnostic: it is a possible forerunner of collagen, very closely related to it, but chemically different; it might be oxidized, or hydrolyzed collagen, or collagen plus or minus some side chain, or an isomer of collagen.

There is little need for discussing these further, with the exception of the definition of Mallory and Parker, which is too categorical and is, apparently, backed by very little chemical proof. If the substances were chemically identical and their differences in staining merely a matter of physical accessibility to the silver-bath, we should expect: (a) that the surface of the section, all of it equally accessible to the fluid, would have a superficial, mirror-like, black

coating, which it does not; (b) that the two substances would be extracted by the same solvents, again untrue; (c) that the action of oxidizing and reducing solutions would affect both in equal degree, which is contrary to the facts.

It should be understood that reticulin and collagen are apparently very closely related from a chemical standpoint and that the differences in their staining reactions, solubility, etc., depend upon presumably trifling differences in their chemical composition — the one seems to glide over into the other in the most gradual sort of transition. For this reason, the statements made in the paper of Mallory and Parker are probably not much overstressed, but their explanation of these phenomena on a purely physical, or mechanical basis seems to be unwarranted.

The origin of these fibers need not detain us here, for we have not been investigating that aspect of the subject; suffice it to say that the precipitation of argyrophil fibrils from a solution that was filtered several times, argues against any but a chemical, or secretory agency on the part of fibroblasts, or other cells of mesenchymal type.

Collagen: Definitions of collagen may be briefly discussed and dismissed.

(a) From the standpoint of the chemist: a substance with a somewhat variable chemical formula and hydrolyzed into gelatin on boiling with water over long periods. It contains no phosphorus.

(b) From the standpoint of the histologist: a tissue component that stains red with acid fuchsin and blue with anilin blue (disregarding its other staining characteristics) and is found in the white fibrous connective tissue.

To elaborate further: the chemist believes that the collagen fiber is gradually broken up and converted into a jelly (gelatin) by hydrolysis, so that his conception of collagen includes the fiber; the histologist believes that any fibrous tissue with the characteristic staining reactions mentioned is collagenous, hence he implies that the whole fiber is composed of collagen, just as the chemist does.

But we have seen that there is, apparently, a more complicated arrangement of the components of collagenous tissue; the fuchsinophilia disappears after boiling the tissue in water without the fibers being destroyed, the collagen fiber apparently gives up a substance with which it has been impregnated, or saturated, without itself going into solution.

CONCLUSIONS

We may, then, speculate on the probable significance of the data set forth in this paper. These would indicate that the connective tissue is composed of a protoplasmic ground substance which is stained by ordinary acidophil dyes such as eosin, picric acid, etc. After extraction with boiling water and weak caustic soda, there remains a stainable framework. After brief extraction with alkaline solution, the collagenous trabeculae and capsule, and the reticulum of the spleen are still fuchsinophil, but the reticulum is no longer argyrophil; after extraction with boiling water alone, this fuchsinophilia disappears, but the argyrophilia remains. Hence it appears that the fibrous and fibrillar framework is composed of one substance and that all this is associated with a water-soluble, fuchsinophilic material; that the reticular tissue contains in addition to this an alkali-soluble argyrophilic substance.

On leaching connective tissue, freed of cellular material by tryptic digestion, in weak NaOH over a considerable period, the fibrous ground substance is also broken up to a greater or lesser extent. Therefore our "reticulin," recovered by chemical isolation, would be a mixture of the protoplasmic ground substance of the fibers *plus* the very scanty argyrophil matter that characterizes reticulum. This is borne out by the silver impregnation, which shows a brownish yellow background of amorphous material in which black granules and (or) threads are demonstrable. Furthermore, in one batch of tissue that was forgotten and left in the NaOH solution in the incubator for four days, the "reticulin" that was recovered showed considerable fuchsinophilia in smears, which indicates that the reticulin had been hydrolyzed by the NaOH into collagen, an assumption quite in accord with the idea that reticulin is a forerunner of collagen. Young tissue shows more of the former, old tissue more of the latter; take cicatrization as an example.

Phosphorus tests on the isolated "collagen" and "reticulin" fractions were very unsatisfactory; the collagen gave a heavy precipitate with ammonium molybdate in acid solution; this dissolved in ammonia, giving a greenish fluid, and reappeared as a bluish green precipitate on adding nitric acid. The reticulin was quite insoluble in acid, unless boiled in concentrated nitric acid; it gave a questionable precipitate with the molybdate, and the addition of ammonia

changed the fluid to a deep orange-brown. This must be worked out more carefully by a skilled chemist. As Siegfried used different methods and as we have seen that our reticulin is apparently a mixture, while our "collagen" is admittedly different from the usual conception of collagen, the presence or absence of phosphorus is relatively unimportant.

There is another point that is not in accord with the usual conception of collagen and gelatin and this is the almost complete failure on my part to obtain any jellies in the aqueous fractions. This might be explained on the basis that the tryptic digestion had removed most of the gelatinous material from the connective tissue; the combination of digestion with very thorough centrifugal washing may be responsible. Once in a while partial jelling of the aqueous fractions would occur, but not often. A review of the literature will show the greatest lack of unanimity on the part of Mall, Young, Siegfried and Tebb on this very question.

In closing, it should be noted that this work is merely a beginning and that there remains a great deal to be done, as the reader has probably already discovered; it is hoped that the results here set forth may interest some skilled biochemist, who will be in a position to prosecute the chemical investigation and identification of the substances isolated by the methods herein described far more successfully than could a pathologist, who lacks the requisite intensive training in biochemistry and the critical chemical judgment that are essential in running down and identifying the various substances involved.

The kindly and ready assistance of various members of the Department of Biochemistry of our College of Medicine has helped the writer over many hard places in this investigation and is most gratefully acknowledged. Messrs. Mènard and Homan have been very coöperative in aiding with the technical details of impregnation and the photomicrography.

SUMMARY

1. In the connective tissue framework of the human spleen, isolated by means of tryptic digestion, there are three main groups of fibrous substances: (a) "collagen," (b) elastin and (c) reticulin.

(a) The first may be completely extracted by boiling water, leaving the white fibrous tissue incapable of taking specific collagen

stains. The extract may be coagulated on slides by means of gentle heat and fixation in Zenker's fluid and a substance will be recovered that gives all the characteristic staining reactions of collagen and many of the precipitation tests of gelatin.

(b) The elastin resists boiling water, and weak acid or alkaline solution. It may be digested with pepsin and HCl, 0.3 per cent. (This was not mentioned in the body of the paper; it is given for the sake of completeness.)

(c) The reticulin is composed of a mixture of at least three groups of substances; an alcohol-soluble group (lipins, largely impure lecithin), an alcohol-insoluble group made up of an argyrophil material that may come down as threads and a silver neutral background of amorphous matter that forms the bulk of the fraction; this may be digested fibers.

2. After extracting these substances from the splenic framework, if alkali-digestion be not carried too far, pale fibers that stain neither with silver nor fuchsin remain; these may be stained with eosin, picric acid, or phosphotungstic acid hematoxylin; rose, yellow, and reddish respectively. ("True collagen?")

3. The staining reactions of the substance extracted with boiling water and those of commercial gelatin are not the same.

4. Using the technic herein described, jellies are very seldom produced.

5. The argyrophil matter in the reticulin is best demonstrated after exposure to certain oxidizing agents, while reducing agents apparently inhibit impregnation.

6. There is a possibility that the water-soluble "collagen" and the alkali-soluble "reticulin" constitute stiffening, strengthening, or protective substances for the fibers of the connective tissue; they impregnate these evenly and may be extracted without destroying the fibers.

7. The assumption that reticulin may be hydrolyzed to collagen is not unwarranted.

NOTE: All but the last two figures represent photomicrographs taken with a 16 mm. objective and 10x ocular.

Photomicrographs by author and Prof. J. B. Homan, of the Department of Medical Art, College of Medicine, University of Cincinnati.

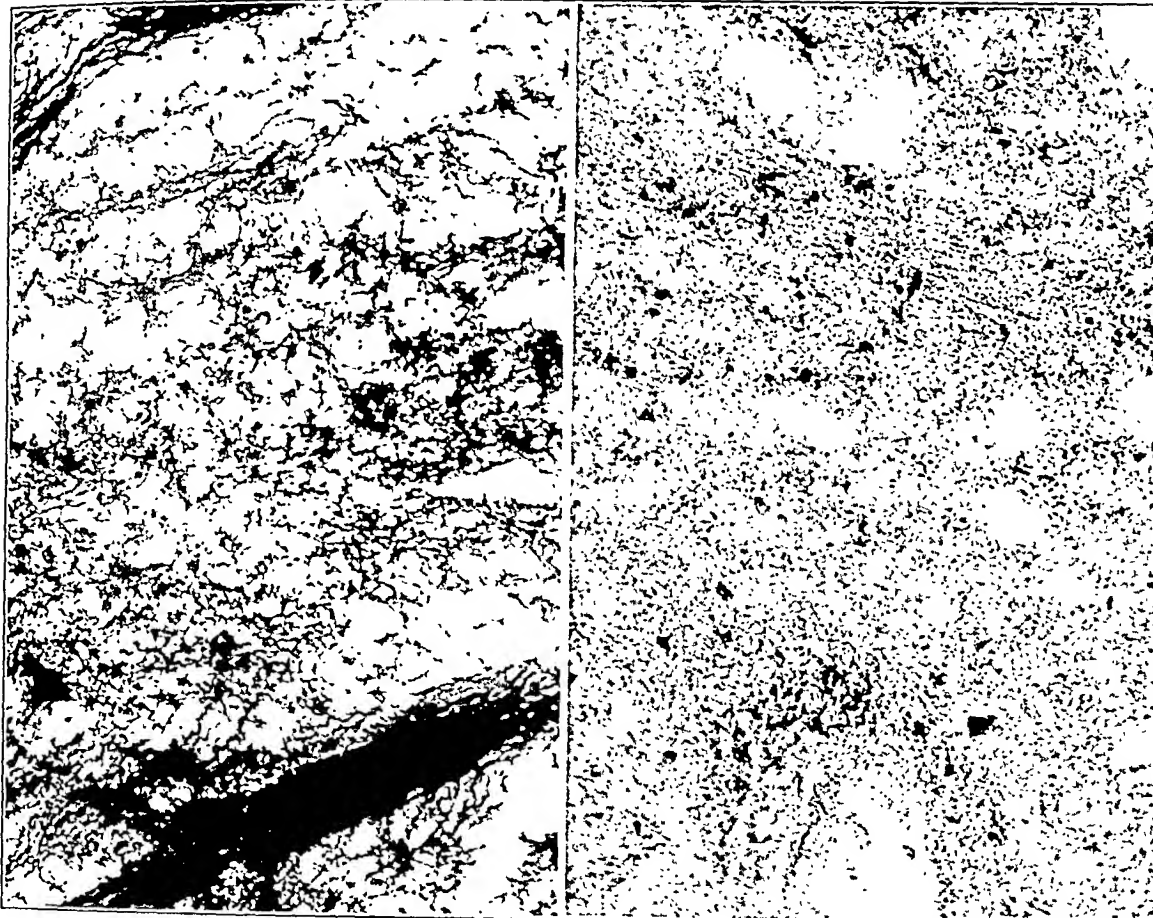
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DESCRIPTION OF PLATES

PLATE 119

- FIG. 1. A frozen section of fresh spleen, subsequently fixed for two hours in absolute alcohol and ether, *aa*. Simple silver impregnation. Reticulum fairly well impregnated.
- FIG. 2. Another frozen section from the same lot, after soaking 20 hours in absolute alcohol and ether. Silver impregnation. Note that the reticulum is no longer well impregnated, although the section is from the same block as the preceding. A Van Gieson hematoxylin stain on a control section from this batch, after 20 hours in alcohol and ether, took perfectly.
- FIG. 3. A paraffin section of water-boiled spleen, silver-Van Gieson technic. The unstained trabeculae should be noted; when they no longer take acid fuchsin they photograph white, instead of black, and the elastic tissue and reticular fibers in them become evident. Notice the masses of coagulated reticulum, well impregnated, in the splenic pulp.



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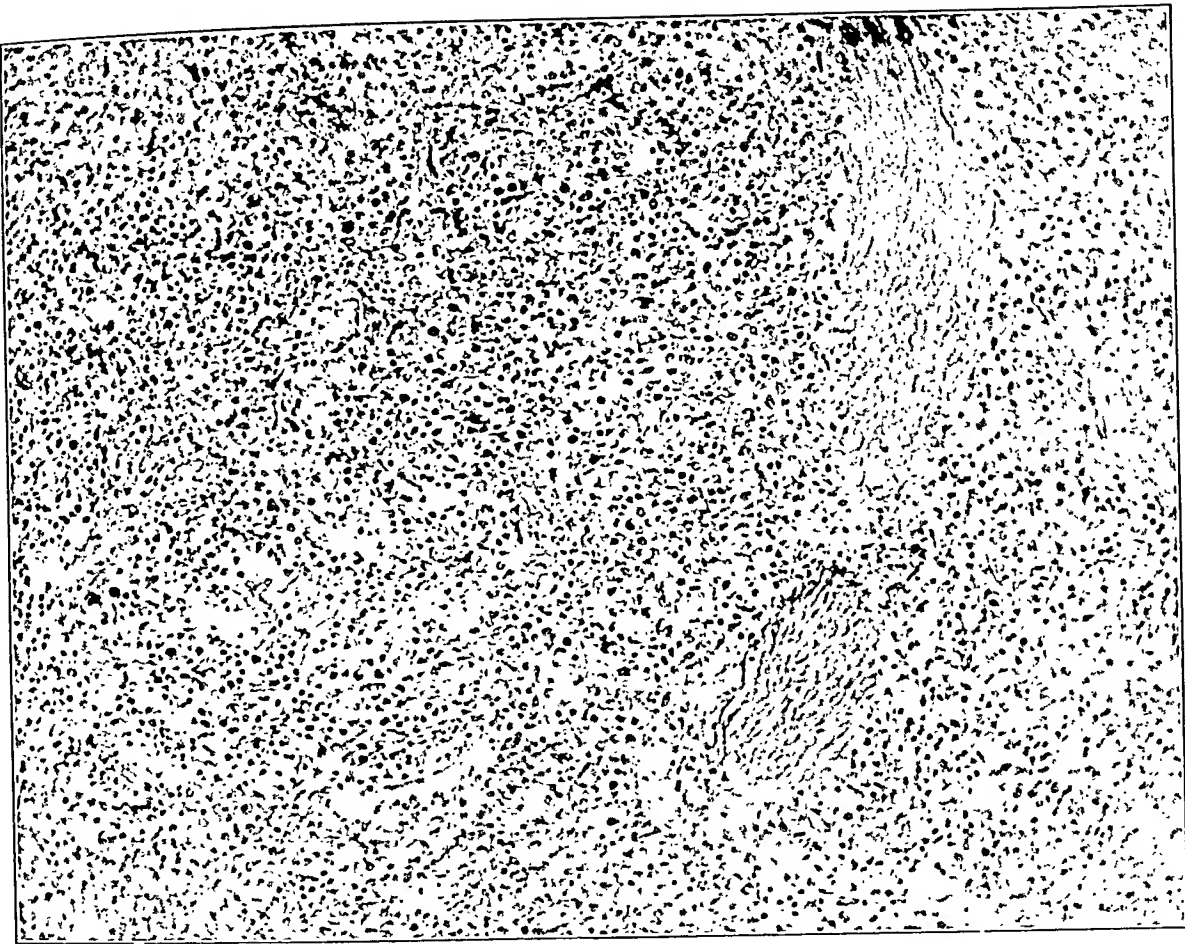
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Collagenous and Reticular Connective Tissue

PLATE 120

FIG. 4. A section of formalin-fixed spleen after silver-Van Gieson impregnation. No pre-treatment with permanganate of potash and oxalic acid. This is an unusually poor impregnation.

FIG. 5. Serial section from same block as Fig. 4 after silver impregnation *preceded* by permanganate of potash and oxalic acid. The difference in the impregnation is self-evident.



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5

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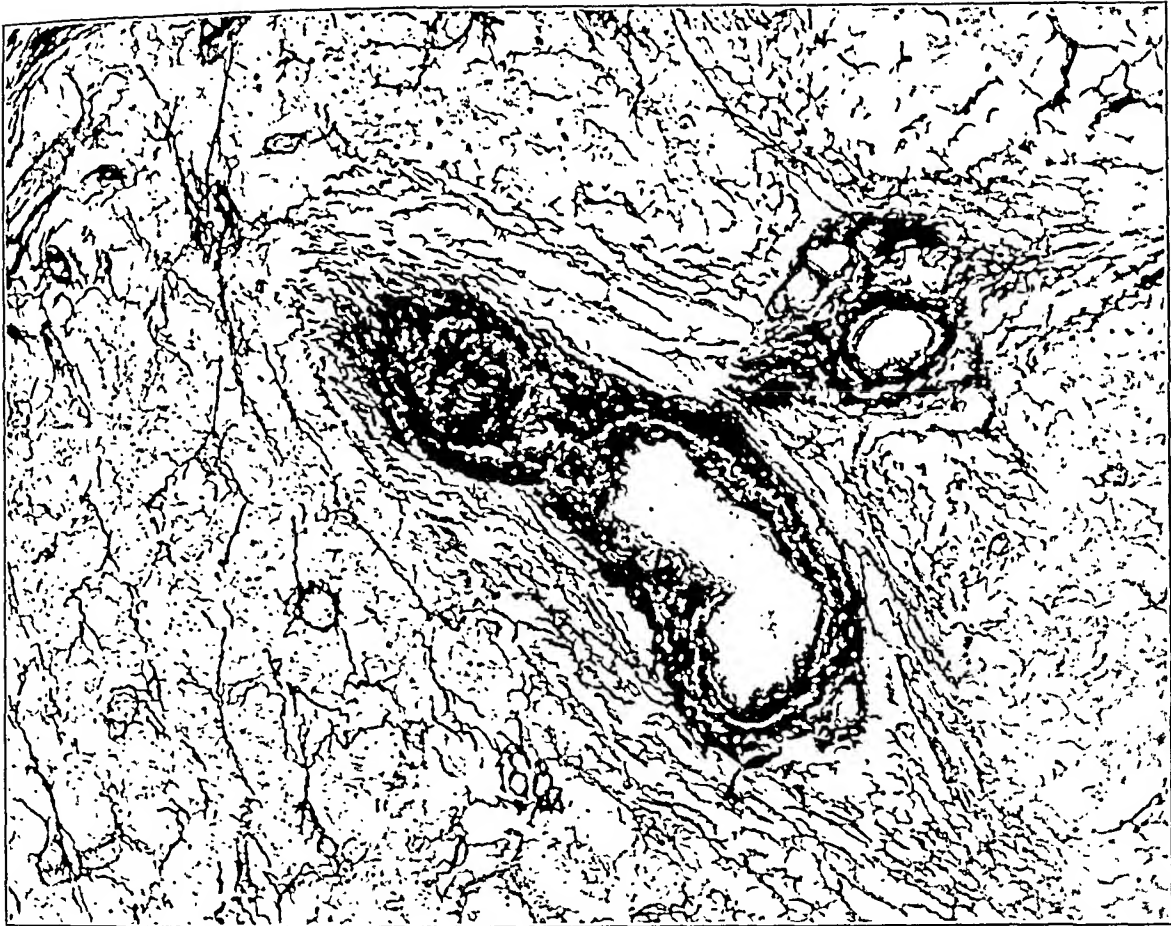
Collagenous and Reticular Connective Tissue

PLATE 121

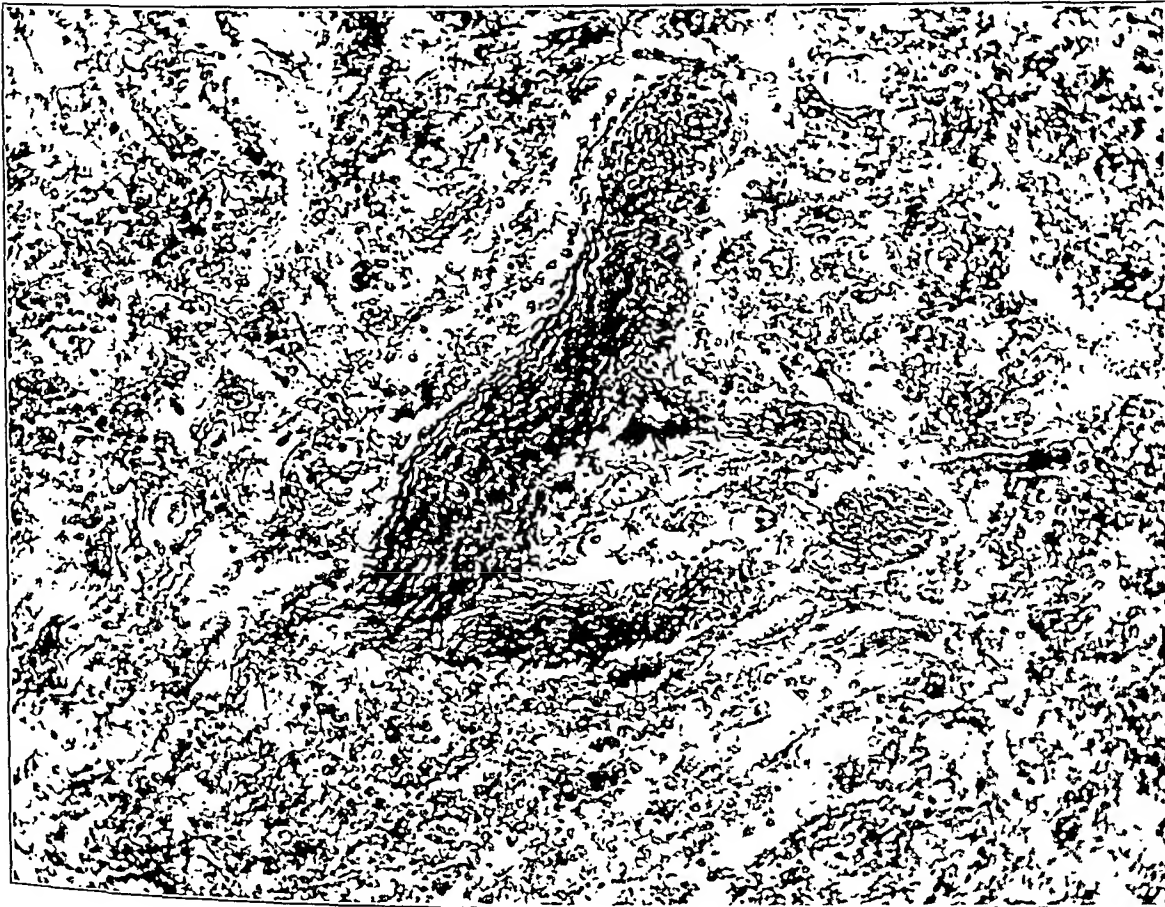
FIG. 6. Same technic as preceding, but from another block of tissue; for comparison with Fig. 7.

FIG. 7. From same block as Fig. 6, with the oxalic acid replaced by 3 per cent hydrochloric acid. Note the totally different type of impregnation resulting from the continued oxidation.

(Figs. 4 to 7, inclusive, were from sections in which the Van Gieson counterstain was omitted.)



6



7

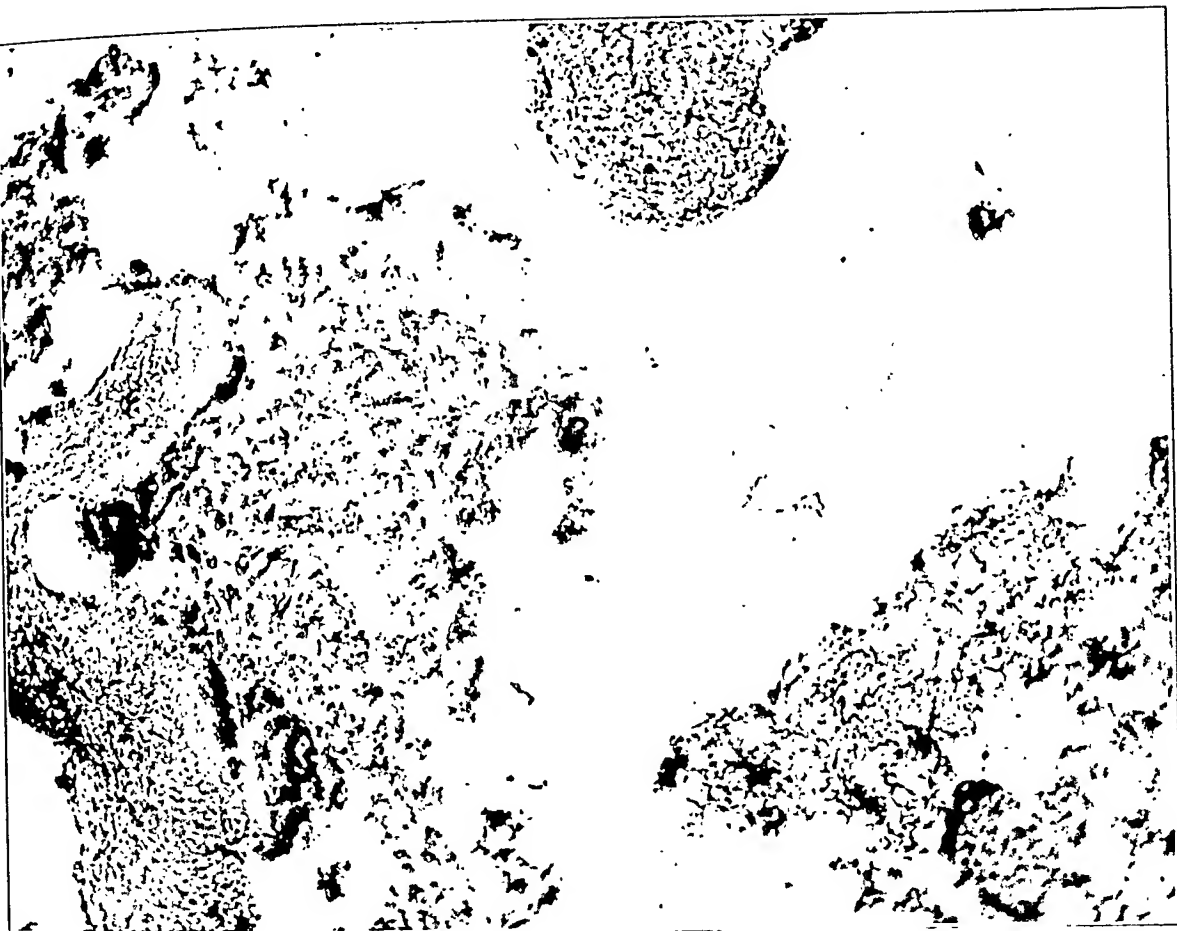
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Collagenous and Reticular Connective Tissue

PLATE 122

FIG. 8. Paraffin section of a mass of precipitate resulting from the boiling of isolated splenic framework with weak HCl. The tissue-like character of the material is perfectly evident. Silver-Van Gieson technic.

FIG. 9. Silver-Van Gieson impregnation of a smear of the precipitate obtained by neutralizing and salting-out the alkaline solution of isolated splenic framework after filtration through filter-paper. The fibers are presumably thrown down by the reprecipitation. Note the disparity in their size and configuration, the beaded appearance of some of them and their general resemblance to reticulum fibers as shown in the preceding figures. The yellowish brown background has been largely eliminated by the yellow-green filter used in taking the photomicrograph.



8



9

Foot

Collagenous and Reticular Connective Tissue

STUDIES IN ACROMEGALY *

VII. THE MICROSCOPICAL STRUCTURE OF THE ADENOMAS IN ACROMEGALIC DYSPIUITARISM (FUGITIVE ACROMEGALY)

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INTRODUCTION

It is the purpose of this paper further to describe a pituitary disorder that appears to be associated with an acidophilic adenoma of foetal type and in which the constitutional evidences of glandular oversecretion are so slight or so masked by signs of glandular insufficiency that they have usually been disregarded. For this disorder we have come to employ the designation *fugitive acromegaly*.

More than twenty years elapsed after Marie¹⁸ gave its name to acromegaly before it was possible to say with any assurance that the hypertrophic features of the malady he had described were actually pituitary in origin and in all probability an expression of glandular overaction. Another twenty years passed before this hypothesis received definite proof through the production first of experimental gigantism in rats (Evans and Long¹³), and more recently of acromegalic gigantism in the dog (Putnam, Teel and Benedict¹⁹) by the parenteral injection of extracts of the anterior lobe.

In the interval the chief arguments in favor of this hypothesis were based almost wholly on the negative evidence of experimental canine hypophysectomies (1908-9) which showed that adult animals (Crowe, Cushing and Homans⁵) became thereby adipose and sexually dystrophic and that young animals (Aschner¹) remained dwarfed and sexually infantile. As these disturbances were obviously due to deprivation of glandular secretion, it was natural to assume that the overgrowth of acromegaly and gigantism represented the opposite state due to an excess of secretion. This assumption received further support from the apparent subsidence in size in the extremities of the first few acromegalic patients that had been successfully operated upon (Hochenegg,¹⁴ Cushing⁹).

* Received for publication August 10, 1928.

Though there was still considerable confusion in regard to the independent function of anterior as opposed to posterior lobe and though the hypothalamic syndrome was as yet unappreciated, on the basis of these experimental and clinical observations the senior author in 1909⁶ ventured to describe pituitary disorders as divisible in a general way into states of hyper- and of hypopituitarism. The existence of intermediary or transitional states was however recognized and in the following year an address on the subject⁸ was concluded with the statement:

"It may be expected: (1) that in all cases of original hyperpituitarism associated with tumor, the functional end-result will be hypopituitarism; and (2) that in many of the cases in which existing hypopituitarism is the striking feature traces at least of an early tendency to hyperpituitarism can be detected. These mixtures of the two syndromes of glandular over- and under-activity are most conveniently designated as *dyspituitarism*."

In a subsequent monograph⁷ dealing with pituitary disorders in general, chief attention was paid to the mechanical effects of intra- and extrasellar tumors of various sorts on the structures in the neighborhood of the gland and but little attention to the histopathological nature of the lesions themselves. As a matter of fact, at that time (1912) the character of the glandular enlargement even in acromegaly was but poorly understood and frequent recourse was had to the non-committal term *struma* in describing it. These "strumas," particularly when associated with hypopituitarism, were recognized as having adenomatous characteristics and to be composed either of chromophobe or of chromophile cells, but in the case of acromegaly it was not clear whether the lesion represented a diffuse functional hyperplasia of the chromophilic elements of the anterior lobe or whether it was actually an adenomatous tumor composed of these elements.

Even Benda,⁴ who in 1901 was the first carefully to study and compare the hypophyseal changes in a series of acromegalics, is somewhat obscure in his description of the lesion which he called *struma hyperplastica* or *adenomatosa*; for though he recognized in three of his four cases "the extraordinary increase of granular (acidophilic) gland-cells" in the lesions, he says that:

"The acromegalic hypophyseal tumors diverge in unmistakable ways from the habits of other diffuse organ-hyperplasias. On the one hand the tumor-character is outspoken even in the smaller tumors by the sharply cut boundary of

the growth; on the other hand histologically the arrangement of the growing cells is neither absolutely like that of the adult nor of the foetal organ. It remains an atypical form which sometimes reaches the border of what we must call malignant."

Unfortunately subsequent investigators were unable to substantiate Benda's views; whether this was from failure to employ sufficiently specific methods of treating the tissues or because the hypophysial "strumas" they chanced to study were not from cases of actual acromegaly is not now possible to determine. The latter is the more probable explanation since more than ten years elapsed before it came slowly to be recognized that by far the most common form of pituitary tumor was unassociated with acromegaly and was a chromophobe type of adenoma which had been regarded previously as a sarcomatous process.

In 1921 Bailey and Davidoff,³ in the study of the tissues which had been secured at operation in this clinic from thirty-five patients with full-blown acromegaly, found, in complete agreement with Benda, that almost without exception they were adenomas whose cells contained acidophilic granules, though in some cases these so-called alpha-granules were present only in small numbers in scattered cells. Four years later, in another paper from this clinic dealing from a general standpoint with pituitary adenomas of all sorts, Dott and Bailey¹² came to the conclusion that not only might an acromegalic show signs of pituitary insufficiency late in the course of the malady but that "in many cases at least, the features of acromegaly and of the hypopituitary syndrome develop synchronously rather than in sequence." They singled out from the series which then comprised 162 verified adenomas, thirteen examples of what, in want of a better term, they called "mixed types," the clinical history of one of the patients having been included (Case VII) in their report.

MATERIAL AND METHODS

In the hope of shedding further light on this obscure "mixed" syndrome, other examples of which have since come under observation, we have undertaken the present study with the purpose of determining whether it might be possible to correlate the degree of eosinophilia found in an adenoma with the coëxistent clinical picture. With this end in view the tissues from a hundred consecutive

adenomas that had been operated on between December 21, 1921, and January 1, 1927, and from which sufficient material for study had been secured and properly fixed, were re-examined. Almost without exception the tumors had attained sufficient size to affect vision by pressure on the chiasm, which was the primary reason for the operation. We are therefore not here concerned with those rare instances of acromegaly, of which Lewis¹⁷ and more recently Krumbhaar¹⁶ have recorded examples, due apparently to a functional hyperplasia of the anterior lobe without actual tumefaction.

The method for identification of the alpha-granules already described by one of us,² of using on Regaud-fixed tissue the neutral dye made by a combination of ethyl-violet and orange-G, was employed throughout.

CLASSIFICATION

From a purely histological standpoint and with complete disregard of the associated clinical symptoms, such a series of adenomas may be divided into six groups with the typical granular chromophilic adenoma of acromegaly at one end of the scale, the typical non-granular chromophobe adenoma at the other end, and with four intermediary types of gradations, as follows:

TYPE I (Fig. 1). This is a highly eosinophilic adenoma which consists of large, polygonal or rounded cells that lie in a loose mass in which there is no stroma of connective tissue and practically no vascular sinuses. The cells which have distinct boundaries vary greatly in size and possess an abundant coarsely granular cytoplasm, often transformed centrally into a hyaline mass so that many nuclei are excentrically situated and crescentic in form. The nuclei are vesicular and show great variability in size. Multinucleated cells are numerous. Crescent cells are also found. *Practically all the cells are packed with alpha-granules, often of large size.*

TYPE II (Fig. 2). This also is a definitely eosinophilic adenoma though of less exaggerated type. The cells have the same sharp boundaries, abundant cytoplasm and vesicular nuclei of varying size. There is no connective tissue stroma and vascular sinuses are rare. The general structure however tends to be more compact so that the cells are largely polygonal and the cytoplasm has rarely undergone the central hyaline change with resultant excentric position and deformation of the nuclei. Multinucleated cells are also less fre-

quently encountered. *Although many non-granular cells are present most of them contain large numbers of alpha-granules.*

TYPE III (Fig. 3). The tumors of this group are those described by Dott and Bailey¹² as "mixed adenomas." Like the above they consist of a compact mass of polygonal cells with well defined boundaries and vesicular nuclei. The cells however have less abundant cytoplasm and multinuclear cells are rarely found. There is no stroma but vascular sinuses are rather numerous. *In the periphery of a variable number of the cells a thin ring of alpha-granules is demonstrable.*

TYPE IV (Fig. 4). The tumors of this group differ from the preceding in that the nuclei are not so consistently vesicular and *no alpha-granules are demonstrable.* Without a specific stain for the granules it would scarcely be possible to distinguish these adenomas from those of Type III.*

TYPE V (Fig. 5). In these tumors there may sometimes be a poorly developed stroma, but the vascular sinuses are fairly numerous and the elongated neoplastic cells sometimes appear to radiate from them. Cellular boundaries are rather indistinct. The relatively scanty cytoplasm of the cells is less deeply stained than in the cells of Type IV and the nuclei are of uniformly smaller size and have a heavier chromatinic network. *No alpha-granules are present.*

TYPE VI (Fig. 6). These adenomas are characterized by a stroma of connective tissue carrying numerous small thin-walled vascular sinuses, dividing the growth into broad cellular columns. The neoplastic cells are elongated or spindle-shaped and often stand at right angles to the septa of connective tissue. They have indistinct boundaries, a feeble staining cytoplasm, and oval, small nuclei containing a heavy chromatinic network. *No alpha-granules are present.*

ATYPICAL CASES. A chromophobe adenoma which is composed of such narrow columns of cells separated by numerous vascular

* Attention should be drawn to the fact that there is some evidence of differentiation in certain of the non-granular cells especially in some of the tumors of this Type IV. If a fragment of such a tumor be immersed in a 1 per cent solution of methyl green and examined under the microscope, the nuclei of the cells will usually be stained bright green and the cytoplasm light yellowish green. However, a variable number of cells, depending on the tumor, will be stained a bright violet and their nuclei remain colorless. The nuclei of these violet cells seem larger than the others. In fixed preparations stained by ethyl-violet, the periphery of these cells retains the dye with considerable tenacity although no definite granules are found. These facts seem to indicate that the chromophobe cells are not all of the same nature.

sinuses that it resembles the normal structure of the anterior lobe is occasionally met with. In those cases of acromegaly also in which there is only a slight enlargement of the hypophysis a somewhat similar structure may be found. These exceptionally rare adenomas have been listed as "atypical."

In the order in which the hypophysial adenomas have been thus subdivided or graded, the cells become smaller, the boundaries less distinct, multinucleated cells disappear, the nuclei become more pycnotic, the cytoplasm less well stained and the stroma and sinuses become more abundant. But more important than all else is the fact that in the descending scale the highly granular cells of Type I have passed to a non-granular type.

CLINICAL CORRELATION

When the hundred adenomas were subdivided on the basis of the foregoing histological differentia without reference to the associated clinical syndrome, the following result was obtained, the most striking feature of the tabulation being the numerical preponderance of the purely chromophobe types.*

Type I.....	8 cases
" II.....	8 "
" III.....	11 "
" IV.....	11 "
" V.....	47 "
" VI.....	12 "
Atypical.....	3 "
Total.....	100

After this histological study had been completed, the voluminous clinical histories were then independently abstracted with special attention to any recorded signs or symptoms that might possibly be interpreted as evidence of a fugitive hyperpituitary effect. It was found that:

1. The sixteen adenomas of Types I and II proved in all in-

* This preponderance of chromophobe cases in the series can in part be accounted for by the fact that the chromophobe adenomas are more likely to impair vision than the adenomas of acromegaly and consequently are for this reason more likely to be operated upon. Though the thick walled sella of acromegaly is capable of distention, comparatively speaking the chromophilic adenomas are less favourable for operative intervention by the usual transphenoidal operation than are the chromophobe adenomas.

stances to have been accompanied by definite clinical acromegaly and in general the more numerous the alpha-cells, the more pronounced was the acromegalic syndrome. To this rule, however, there were the following exceptions: In six of the cases with outspoken acromegaly numerous alpha-cells had been demonstrated in the tissues, but they seemed to be fewer than the intensity of the clinical picture would have led one to expect. There were, however, in these cases some extenuating circumstances. In two of them the tissues were fixed in Zenker's fluid and it is possible that more alpha-cells were present than could be demonstrated. In two other cases there was evidence that the acromegalic process was inactive at the time of the operation.

2. The twenty-two transitional adenomas of Types III and IV with which we are chiefly concerned proved to be associated in many instances with clinical indications of an abortive hyperpituitary action. This was almost invariably true of the eleven Type III adenomas which showed occasional cells with a peripherally disposed thin ring of alpha-granules. In this group enlargement of hands and feet was recorded in six instances; coarsening of the features in eight, hypertrichosis in two, normal or exaggerated libido in two, excessive height in three, excessive perspiration in two, squaring and tufting of the phalanges in four, persistent lactation in one, glycosuria in one, and four of the cases had a normal or slightly elevated basal metabolic rate whereas practically without exception the rate in all patients with adenomas of Types IV, V and VI was subnormal, often markedly so.

Among the eleven patients with Type IV adenomas, although no alpha-granules were demonstrable, two prove to have been very tall and heavily built, one had spacing of the teeth, one hypertrichosis and exaggerated libido and another definite tufting of the terminal phalanges.

3. The fifty-nine patients with purely chromophobe adenomas of Types V and VI had given no clinical evidence of anything suggesting even a trace of fugitive hyperpituitarism with three possible exceptions: In two patients with Type V lesions, a suggestive tufting of the terminal phalanges had been mentioned in the X-ray report; and one patient with a Type VI lesion was a tall man with prominent lower jaw but no malocclusion of the teeth. Whether these trifles can be looked upon as suggesting a taint of acromegaly is doubtful.

As a result of this clinico-pathological study it may be stated with reasonable conservatism: (1) that highly acidophilic adenomas are invariably accompanied by unmistakable acromegaly; (2) that the pure chromophobe types are with equal definiteness unassociated with any clinical traces of this disorder; and (3) that an intermediary group of adenomas with sparsely disposed cells containing a peripheral zone of alpha-granules, are likely to produce clinical evidences of a fugitive hyperpituitary reaction.

The histological characteristics of the adenomas of this intermediary group are in other words, sufficiently distinctive to enable the pathologist to state, without knowledge of the clinical symptoms, that the patient probably shows traces of hyperpituitarism. What is more, it is possible for the clinician to predict with some assurance when this intermediary type of adenoma will be disclosed by the microscope. Such a clinical prediction has received histological verification in four cases operated upon during the past year.

CASE REPORTS

It will suffice to give a few briefly abstracted case histories in illustration of the condition that we have come to designate a transitional, abortive or fugitive acromegaly.

CASE I

Transitional Adcnoma of Type III with Marked Local Symptoms and Dyspituitarism Chiefly Shown by Adiposity in a Man of Large Frame

On September 18, 1923, Harry P. (Surg. No. 19695), aged 24, single, a foreman, was referred to the hospital by Dr. L. Solis Cohen of Philadelphia with the diagnosis of pituitary tumor.

History: Prior to 1918, he had always been well. In that year he began to be troubled by indistinct vision, supposedly astigmatic. Shortly afterward he began to gain rapidly in weight from 170 to 220 pounds. During the past six months vision has failed to such an extent that he is no longer able to read print. His secondary sex characters appeared at a normal age; he has always been somewhat sparsely bearded; libido has been undiminished during the present illness. There has been no polyuria or polydipsia, no headache, no loss of mental vigor.

Examination: This disclosed a bilateral primary optic atrophy, a complete bitemporal hemianopsia, and a greatly enlarged and deepened sella turcica. The basal metabolic rate was -5 per cent. No glycosuria, no polyuria.

The patient was a heavy-boned, rather obese young man (Figs. 7 and 8), 5 ft. 10 in. tall and weighing 225 pounds. His skin was smooth and hair was scanty over face and chest. His extremities were disproportionately large. He wore a 7 $\frac{3}{8}$ hat, a 11 $\frac{1}{2}$ shoe and No. 9 glove. The features were coarse and the

lips thick; there was maxillary rather than mandibular prognathism. The cranial X-ray showed the accessory nasal sinuses to be considerably enlarged. The external genitalia were normally developed.

Clinical Impression: Hypophysial adenoma with dyspituitarism.

Operation: On September 27, 1923, the transphenoidal approach to the distended sella proved to be difficult because of ossification of the cartilage of the septum, and the presence of hypertrophic spurs such as occur in acromegaly. The exposure of the distended sellar floor was consequently difficult. About a thimbleful of soft adenomatous tissue was removed.

Course of Illness: There was no immediate postoperative improvement in the visual fields but the acuity increased from 20/200 to 20/50 and he was discharged on October 4, 1923. He was given a series of X-ray treatments with further improvement in vision which has been retained for five years. When last heard from on June 29, 1928, he reported himself to be in excellent health and weighing 20 pounds less than in 1923, so that his malady may be considered stationary.

Microscopical Examination: Fixatives: Regaud, Zenker. Stains: M.V.E., P.T.A.H., E.V.O.G. The tumor is composed of polygonal and elongated cells with abundant granular cytoplasm (Fig. 9a). The nuclei are large and vesicular. An occasional multinucleated cell is seen. Vascular sinuses are rare and there is no tendency to form a stroma of connective tissue. With E.V.O.G. stain a few cells are seen with blue granules in the periphery of the cytoplasm (Fig. 9b).

Pathological Diagnosis: Transitional adenoma of Type III.

Comment: Although this patient might have passed in 1923 as having a mild hypopituitary syndrome, the fact of his unusually large extremities with a heavy skeleton aroused the suspicion that there might be an acromegalic taint and this was further supported by the hypertrophic condition of the nasal septa encountered at the operation. The pathological diagnosis at the time of the operation was that of pituitary adenoma (unqualified) and its foetal eosinophilic type was not recognized until the tissues were more recently reviewed.

CASE II

Large Transitional Adenoma of Type III with Dyspituitarism in an Adipose Woman of Large Frame

On November 3, 1924, Ida H. (Surg. No. 22521), a farmer's wife, 37 years of age, was referred to the hospital by Dr. William R. Goff of Parkersburg, West Virginia, with the diagnosis of pituitary tumor.

History: The patient had been a vigorous woman though susceptible to recurring attacks of "quinzy" until her tonsils were removed in 1921. After bearing three healthy children her menses ceased. This occurred in 1914, ten years before entrance, and was ascribed to a preceding attack of typhoid fever, after which she began to grow stout (135 pounds in 1914 to 193 pounds on admission). In 1922 she began having bitemporal headaches with progressive obscuration of vision. She has suffered of late from indigestion, dyspnoea, sensitivity to cold and fatigability with weakness. She has noticed an increasing coarseness of her features and increase in size of her hands and feet.

Examination: This showed a large, obese woman of middle age with heavy features (Figs. 10 and 11) and large hands and feet. The skin was coarse and damp with perspiration. There was pallor of the optic nerves, bitemporal field defects, and a greatly enlarged and deepened sella turcica. The X-ray films of the hands showed "suggestive squaring of the metacarpals but no other evidence of acromegalic change." There was no glycosuria nor polyuria. The basal metabolic rate was +5 per cent. The temperature tended to be subnormal.

The *clinical impression* was that of a hypopituitary state with traces of antecedent acromegaly.

Operation: On November 14, 1924, a transphenoidal operation was performed with intrasellar removal of considerable soft adenomatous tissue which was under tension and comparatively avascular. She made a good recovery with both subjective and objective improvement in vision. She was given subsequent X-ray treatments and was discharged on December 4, 1924. No subsequent reports.

Microscopical Examination: Fixatives: Zenker, Regaud. Stains: M.V.E., P.T.A.H., E.V.O.G. The tumor is composed of polygonal cells with rather abundant, coarsely granular cytoplasm (Fig. 12a). The nuclei tend to be vesicular and often excentrically situated. Multinucleated cells are occasionally seen. Small vascular sinuses are present but there is no tendency to form a stroma. With E.V.O.G. stain a few scattered cells are seen which contain fine blue granules in the periphery of their cytoplasm (Fig. 12b).

Pathological Diagnosis: A transitional adenoma of Type III.

Comment: It would appear from the clinical record that the patient was suspected of having dyspituitarism associated with a transitional or fugitive acromegaly though these descriptive terms had not yet come into use. The pathological diagnosis of the lesion, based on routine examination, was again "pituitary adenoma" (unqualified) and it was not until our recent reclassification on the basis of secretory granules that the suspicion of a transitory hyperpituitarism received histological support.

CASE III

Dispituitarism with Transitional Acromegaly in a Man with a Large Adenoma of Type III that had Extruded into the Sphenoidal Cells

On December 17, 1924, Harold P. (Surg. No. 22822), aged 44, a steamfitter, was referred to the hospital by Dr. William F. Regan of Boston with the complaint of headaches and numbness of extremities.

History: The patient was married and the father of one child 16 years of age. Previously of vigorous health, he for ten years had been having recurring headaches, sometimes so severe they caused vomiting and profuse sweating, obliging him to lay off from work. After a period of remission for a few years, these headaches have returned though in a milder form. During the past three or four years he has gained 50 pounds in weight and there has been a noticeable diminution of energy and ambition together with loss of libido. Meanwhile his vision has been noticeably failing. He has been on an antiluetic treatment.

Examination: He was a powerfully built, ponderous and obese man (Figs. 13 and 14) over six feet in height and weighing 211 pounds. The skin was soft and smooth with scanty axillary and pubic hair and sparse, slow growing beard so that he shaved only once a week. The genitalia were small. The hands and feet were big and broad and though the fingers tapered normally the X-ray disclosed slight tufting of the terminal phalanges. There was no prognathism but the frontal and sphenoidal sinuses were large. The sella was huge, with pressure destruction of the posterior clinoid processes. There was a bilateral choked disc of low grade and left homonymous field defects. The temperature tended to be subnormal; the basal metabolic rate was -11 per cent. No glycosuria or polyuria.

Clinical Impression: Hypophysial adenoma with hypopituitarism and traces of antecedent acromegaly.

Operation: On January 5, 1925, a transphenoidal operation was performed in the course of which an hypertrophic maxillary spur such as characterizes acromegaly was encountered. An abundance of soft adenomatous tissue which had already extruded through the sella and was filling the sphenoidal cells was radically removed. The recovery from the operation was uneventful but the headaches continued and the left homonymous hemianopsia progressed. He was given a course of X-ray treatments and was discharged on January 29, 1925.

Course of Illness: He reported on May 29, 1925, when his visual fields were found to have filled out almost to normal, with a slight residual left upper quadrantal defect. His general condition was much improved and he had gone back to work. On February 16, 1926, he was found to have again become apathetic and the defect in his visual fields was larger. Another series of X-ray treatments was given. When last heard from, July 3, 1928, it was reported that the patient had had two or three attacks which seemed like convulsive seizures. Otherwise his condition was largely unchanged and he was able to continue with his work.

Microscopical Examination: Fixatives: formalin, Regaud, Zenker. Stains: M.V.E., P.T.A.H., E.V.O.G. The tumor is composed of polygonal and irregular cells with abundant, coarsely granular cytoplasm and sharply defined boundaries (Fig. 15a). The nuclei are large and vesicular. A few multinucleated cells are seen. There is no stroma, but vascular sinuses are numerous and in some areas there is a large amount of collagenic tissue, probably secondary to degenerative change in the tumor. With E.V.O.G. numerous cells have a ring of fine blue granules in the periphery of the cytoplasm (Fig. 15b).

Pathological Diagnosis: Transitional adenoma of Type III.

Comment: This man obviously had a sufficiently large intracranial extension of his adenoma to cause an increase in intracranial tension probably due to mild hydrocephalus with resultant papilloedema. The tumor had also broken through the floor of the distended sella so that the sphenoidal cells were filled with the growth. Examples of similarly extensive adenomas in cases, however, of unmistakable acromegaly have been recorded by Cushing and Davidoff in a previous paper in this series.¹⁰ In this particular case the mixture of clinical hypo- and hyperpituitary symptoms was sufficiently definite to arouse the suspicion that an adenoma of transitional type

would be disclosed. This prediction was upheld by the histological studies of the tissues.

CASE IV

Large Transitional Adenoma with Marked Neighborhood Symptoms and Dyspituitarism with Fugitive Hyperpituitarism Shown by Glycosuria

On *Sept. 30, 1925*, Robert P. (Surg. No. 25120), a civil engineer, 33 years of age, entered the hospital on the advice of Dr. Robert B. Giles of Dallas, Texas, with a diagnosis of pituitary tumor which had been long treated by antiluetic measures and by radiotherapy. The sole complaint was of failing vision.

History: Shortly after the patient's discharge from the Army in 1919, he noticed a periodical, bitemporal clouding of vision which by *December of 1923* had markedly increased. In 1924 he began to have fronto-temporal headaches and to gain in weight, which increased from his normal of 125 pounds to 183 pounds by January of 1925.

In 1923, owing to a supposedly positive Wassermann, he was given a vigorous antiluetic treatment; and in July of 1925, by which time his vision had become markedly impaired, he was given X-ray treatment in spite of which vision continued to fail.

Examination: He was a well developed, over-well nourished man 176.3 cm. in height (Figs. 16 and 17). There was considerable puffiness of the face and coarseness of the skin associated with a moderate hypertrichosis. The genital development and libido were normal. Though married he had no children. There was no evidence of skeletal overgrowth either of skull, hands or feet shown by the X-ray. The basal metabolic rate was -18 per cent.

The neighborhood symptoms were pronounced: the sella greatly enlarged; bilateral primary optic atrophy; a temporal hemianopsia in the left eye with 20/100 vision and vision in the right eye reduced to shadows. The patient brought with him a perimetric chart taken three months previously at which time there was a fairly typical bitemporal field defect.

The case would have been looked upon as an example of probable chromophobe adenoma with hypopituitarism had it not been that he was found to have glycosuria, the urine showing 1.42 per cent of sugar.

He had come across the country by automobile and arrived with a most severe case of hemorrhoids which more urgently demanded attention than his hypophysial tumor in spite of his approaching blindness. The hemorrhoids were operated upon on *October 5, 1925*, and from this procedure he made a speedy recovery. He was re-admitted to the hospital a month later.

Operation: On *November 13, 1925*, the usual transphenoidal operation was performed in the course of which it was found that the tumor had extruded into the sphenoidal cells. In consequence of this a much less satisfactory and thorough extirpation of the growth than usual was made and it was not certain how much of the intrasellar portion of the growth had actually been removed. He made an excellent postoperative recovery but unfortunately without the usual marked improvement in vision. However, he subsequently did well and on *July 14, 1927*, he reported that he had not missed a day at his office and was enjoying good health.

Microscopical Examination: Fixatives: Regaud, Zenker. Stains: M.V.E., P.T.A.H., E.V.O.G. Tissue removed consists of a portion of flattened anterior

lobe and small fragments of adenoma. The latter is composed of polygonal cells with abundant, coarsely granular cytoplasm (Fig. 18a). Blood vessels are frequent and there is a tendency to form a stroma. The nuclei are vesicular and a few multinucleated cells are seen. E.V.O.G. stain shows numerous cells which contain blue granules (Fig. 18b).

Pathological Diagnosis: Transitional adenoma of Type III.

Comment: In spite of his glycosuria, this patient was looked upon as a typical example of mild hypopituitarism and the adenoma had been recorded as chromophobe in type until it was found during our recent re-examination of the tissues that many of the cells had peripherally disposed of granules.

In a previous number of this series of studies,¹⁰ it was stated by Davidoff and Cushing that whereas one out of every four patients with acromegaly shows glycosuria at one time or another in the course of the disease, patients with pituitary insufficiency on the contrary almost invariably show a high carbohydrate tolerance. The presence of glycosuria in this patient should consequently have sufficed to make us hesitate to accept the original clinical and pathological diagnoses that were made. The subsequent disclosure in this case of an adenoma whose cells contain alpha-granules is consequently of particular interest and may possibly afford an explanation of other cases that have been reported in which glycosuria has been observed in patients with supposed hypopituitarism.

OTHER CASES

It would merely prolong without strengthening this communication should we submit to the temptation of giving the clinical histories of the remaining seven patients in whom a Type III adenoma was found. In all of them the diagnosis of chromophobe adenoma had originally been made and yet, the notes on most of the histories make it apparent that the clinical observers were puzzled by the presence of symptoms that are uncommon in pure hypopituitary states. The usual syndrome was that shown by the first three of the four patients whose histories have been given, namely, suggestive signs of a tendency to overgrowth; but acromegaly in Marie's sense, though the most striking feature of adult hyperpituitarism is by no means its only manifestation. Three of the patients were women who were victimized by an offensive degree of hypertrichosis in association with an exaggerated libido. Another woman following

second pregnancy failed to regain her menstrual function and has continued to lactate for a period of six years; she shows coarsening of the features, is troubled by excessive hyperhidrosis and has gained thirty pounds in weight. The majority of these patients had a basal metabolic rate estimated at slightly above zero per cent, whereas a rate of -20 to -30 is usual in uncomplicated hypopituitarism associated with chromophobe adenomas.

The anterior lobe may possess a dual function with independent principles influencing growth on the one hand and the organs of sex on the other. Time will shed light on this. Meanwhile it is not at all unlikely that failure in the past to recognize fugitive expressions of hyperpituitarism, that are unaccompanied by any clinically demonstrable evidences of skeletal overgrowth, may have been a source of confusion. The cases described in the literature (*e.g.*, Kraus,¹⁵ Case 23) in which an eosinophilic adenoma has been unaccompanied by acromegaly may come to be thus explained.

DISCUSSION

We are aware that the histological studies of the tissues on which our type-grouping has been based are open to criticism on two scores: (1) in that only a small part of the tumor has been subjected to examination and the assumption must be made that the portion removed is representative of the entire growth; and (2) in that the tumors vary in size so that there is no means from the operative specimen of estimating the total number of alpha-cells in a given lesion. In spite of these possible sources of error the correlation between the clinical syndrome and the microscopical structure of the adenoma seems to us to be sufficiently consistent to justify the conclusion that *there is a definite relationship between the number of alpha-granules in a hypophysial adenoma and the intensity of the associated acromegalic syndrome.*

In an attempt, from the standpoint of functional pathology, to interpret the clinico-pathological significance of this association of transitional adenomas with fugitive acromegaly, we may begin with the one fact concerning overgrowth that now has an experimental basis: namely, that the prolonged administration of extracts of the anterior lobe of the hypophysis can produce states in animals comparable to gigantism and acromegaly in man. Almost certainly,

therefore, these latter conditions are due to hypersecretion rather than to a possible perversion of secretion of the cells of the anterior lobe.

Since acromegaly is ordinarily accompanied by a tumor which compresses and seriously compromises or may even destroy the normal hypophysial tissue, the continuous pathological overgrowth must be due to the secretory activity of the tumor itself. This assumption is strengthened by finding that the cells of the tumor contain the same granules that are present in the normal eosinophilic cells of the anterior lobe. The further assumption is therefore justifiable that these alpha-granules represent the secretory product that has to do with growth.

We are further struck by the fact that beta- (basophilic) granules are never found in hypophysial adenomas. It is true that basophilic adenomas of microscopical size have been reported by Erdheim and others, but we have never found, by the use of specific stains, beta-granules in the cells of any of the two hundred or more adenomas that have been carefully examined. Though adenomas are occasionally described as basophilic, this is probably due to the fact that the cells of the ordinary chromophobe adenoma stain somewhat more heavily with hematoxylin than with eosin. We have no clue as to the significance of the beta-granules, either from the study of adenomas or from any other source. Whether they are in any way related to the alpha-granules is a subject of dispute.

The chromophobe cells of the anterior lobe are supposed to be more embryonic in type than the chromophile cells and this supposition seems in accord with the fact that the chromophobe adenomas are almost always accompanied by symptoms of lowered hypophysial function and never by symptoms of overfunction. They appear, moreover, to be in general more rapidly growing than the eosinophilic adenomas.

While it is true that the cells of acromegalic adenomas contain alpha-granules, yet they have not the exact structure of normal eosinophilic cells and great numbers of them contain no granules. It is difficult to say whether these non-granular cells are embryonic chromophobe cells or eosinophilic cells which have lost their secretory activity. Kraus,¹⁵ believing that certain non-granular cells of the normal anterior lobe are chromophile cells which have lost their granules, speaks of them as "*entgranulierten Zellen*." On the other

hand, as we have already noted, most of the chromophobe cells of the normal hypophysis appear to be of an undeveloped and more or less embryonic type.

The possibilities are several: (1) that the cells of the adenomas begin as chromophobe elements and subsequently acquire their acidophilic granules; (2) that they originate as alpha-cells and later lose their granules; or (3) that the cells in a given tumor, whether chromophile, intermediary or chromophobe, remain of the same type from the beginning to the end of the process.

Although we have occasionally had the opportunity of studying specimens from a given adenoma taken at different stages of the disease, we have never observed any essential change to have taken place in its cellular characteristics; as a matter of fact, surgical specimens are rarely secured from these tumors in other than the late stage of their development. We accordingly are shut off from employing this direct method of observing cytological alterations that may possibly occur in the tumor as its growth progresses, and are obliged consequently to turn to indirect evidence in discussing these hypotheses.

In accordance with the first hypothesis we would expect the patient to show primary signs of hypofunction on which evidences of acromegaly are subsequently grafted; if the clinical records are to be relied upon, this seems actually to have happened in two of our cases, but it must be exceedingly rare. The symptomatic converse, which would accord with the second hypothesis, namely to see signs of ultimate pituitary insufficiency in a patient primarily acromegalic, is, on the other hand, far from uncommon. But experience now teaches us that there are other patients with obvious dyspituitarism who show fugitive symptoms of acromegaly from the beginning to the end of their disease.

It is in the adenomas of patients in this last category that the cells containing only a ring of fine alpha-granules in the periphery of the cytoplasm are to be found. And since these cells have a close resemblance to the young alpha-cells as they first appear in the embryo (Fig. 19) it is reasonable to suppose that they have never attained maturity and that they remain of this embryonic type throughout the course of the disease.

In further consideration of the second hypothesis it is interesting to consider the structure of the adenoma of those cases of well de-

veloped acromegaly, which at the time of operation have apparently passed into a phase of hypofunction (as evidenced by a low basal metabolic rate, hypotrichosis, etc.) in the absence of cystic degeneration, hemorrhage into the tumor or other destructive lesion which might account for the change in the clinical syndrome. One would expect from the general structure of the adenomas in these cases (*i.e.*, rounded cells with central hyalinization, crescentic excentric nuclei, numerous multiloculated cells, rare vascular sinuses, etc.) that all of the cells would prove to be crowded with alpha-granules. But as mentioned (*cf.* p. 551) among the exceptions to the rule that the more numerous the alpha-cells the more pronounced was the acromegaly, numerous cells are found in these transitional cases which are entirely free from granules. On the basis that these non-granular cells were probably once eosinophilic and now functionless (*entgranulierten Zellen* of Kraus), it is possible to understand the change of the clinical syndrome occurring in these cases.

These of necessity are purely theoretical considerations and when one recalls how long a time we have been in coming to an understanding of the nature of the adenomas of the thyroid gland which are far more accessible for study, impatience to get at the truth regarding the cytological evolution of the hypophysial adenomas may well be restrained. Meanwhile in concluding this report it is safe to say in:

SUMMARY

That whereas a highly chromophilic type of adenoma, whose cells are heavily laden with alpha-granules, characterizes outspoken acromegalic hyperpituitarism, and whereas an adenoma of purely chromophobe type with non-granular cells is commonly associated with adult hypopituitarism, there is an intermediary group of cases in which traces of these opposed symptoms have apparently been present from the outset and which are associated with a histologically distinctive adenoma with cells of foetal type having sparse, peripherally disposed granules.

Since this intermediary syndrome is distinguishable clinically from the more common hypopituitary state by recognizable traces of hyperpituitarism, we find it convenient to refer to the disorder as *fugitive acromegaly*.

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DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Chromophile adenoma of Type I. (*a*) The cells of variable size, often multinuclear, lie in a loose, practically non-vascular mass (hematoxylin and eosin, $\times 300$). (*b*) Practically every cell is packed with alpha-granules (ethyl violet-orange G, $\times 850$).

FIG. 2. Chromophile adenoma of Type II. (*a*) The tumor is more compact: one multinucleated cell is seen; the nuclei are more uniformly spherical and vesicular (hematoxylin and eosin, $\times 300$). (*b*) Many cells contain abundant alpha-granules in the periphery of the cytoplasm (ethyl violet-orange G, $\times 850$).

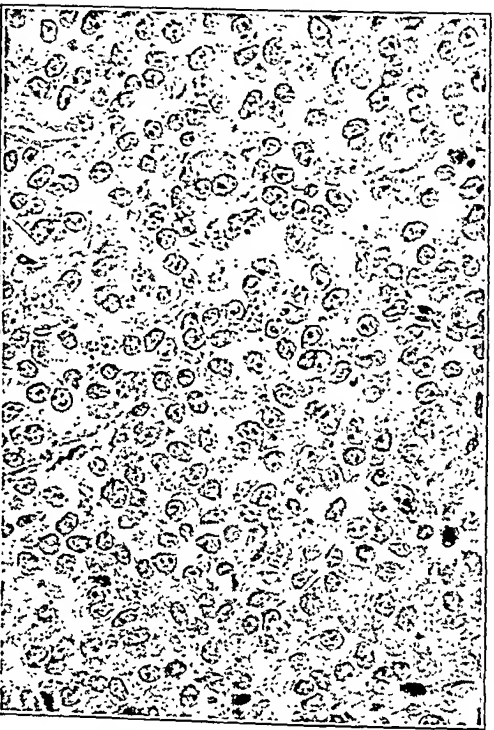


a

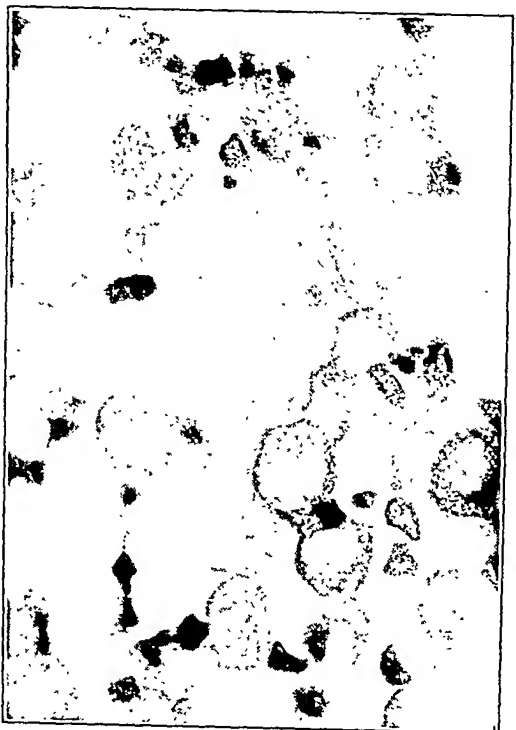


b

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a



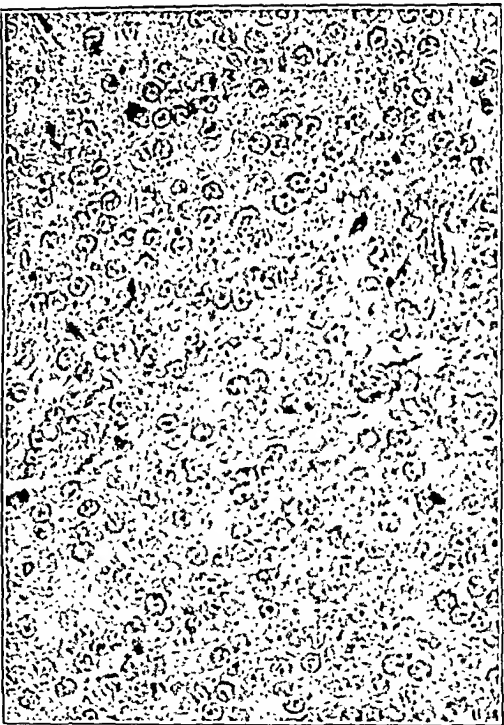
b

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PLATE 124

FIG. 3. Transitional adenoma of Type III. (*a*) The cells have vesicular nuclei with less abundant cytoplasm. No multinucleated cells. Few vascular sinuses. (Hematoxylin and eosin, $\times 300$.) (*b*) Note the dark ring formed by alpha-granules in the peripheries of the cells (ethyl violet-orange G, $\times 850$).

FIG. 4. Chromophobe adenoma of Type IV. (*a*) The tumor is similar to Type III though the nuclei are less consistently vesicular (hematoxylin and eosin, $\times 300$). (*b*) No alpha-granules. Only the nucleoli and erythrocytes are stained (neutral ethyl violet-orange G, $\times 850$).

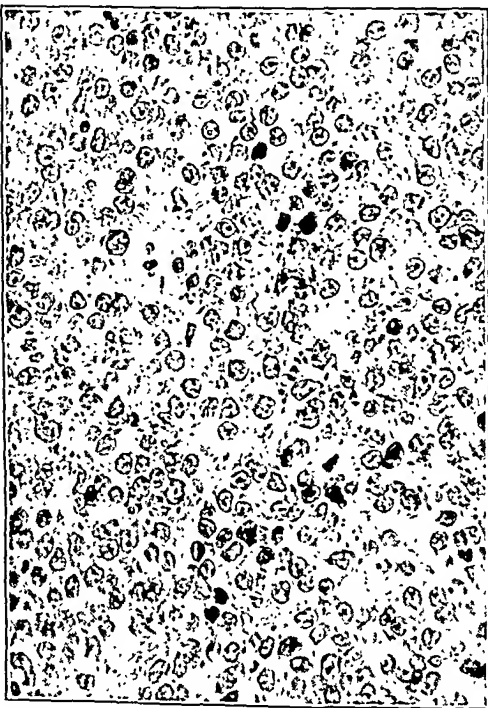


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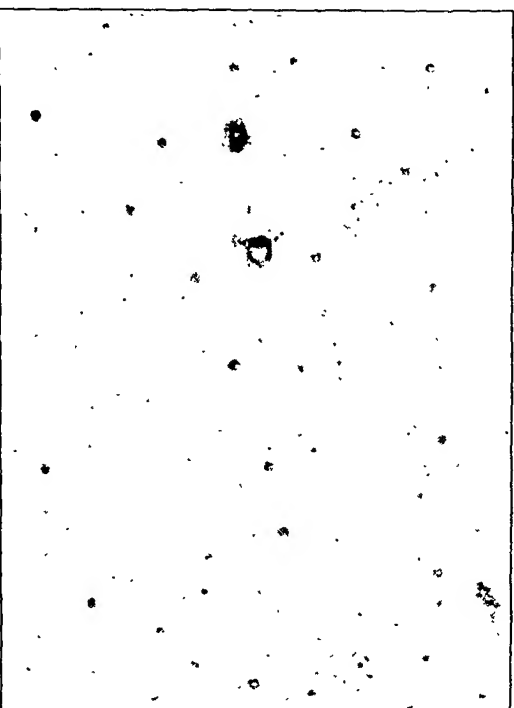


b

3



a



b

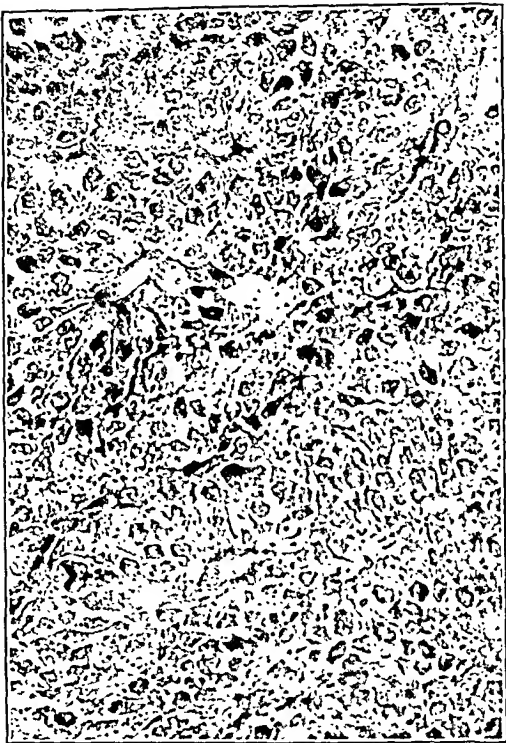
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PLATE 125

FIG. 5. Chromophobe adenoma of Type V. The poorly defined cells show a tendency to radiate around numerous small vascular sinuses (methylene blue-cosin, $\times 300$).

FIG. 6. Chromophobe adenoma of Type VI. The broad columns of cells whose nuclei are non-vesicular are separated by a stroma of connective tissue holding vascular sinuses (hematoxylin and eosin, $\times 300$).

FIGS. 7 and 8. Case I. Note the large hand, thick lips and nose, coarse skin and prominent maxillary region.



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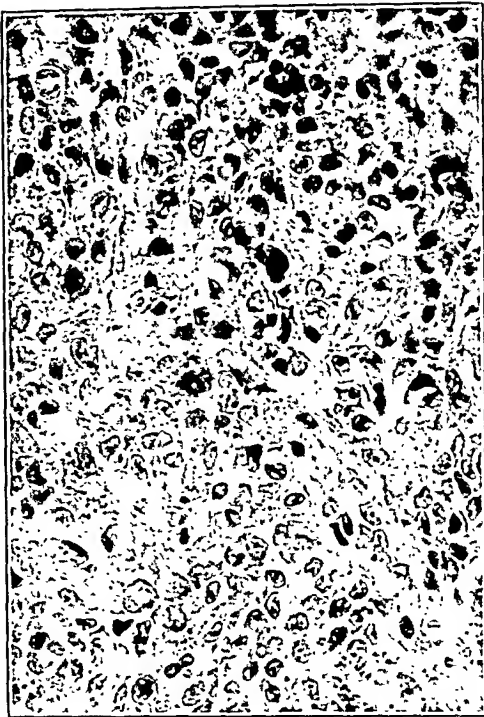


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PLATE 126

FIG. 9. Case I. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, $\times 300$.) The nuclei have a tendency to be vesicular. (*b*) (Neutral ethyl violet-orange G, $\times 850$.) Two cells may be seen with a rim of alpha-granules.

FIGS. 10 and 11. Case II. Slight obesity with coarsening of the skin and features. Hand fairly normal.



a



b

9



10

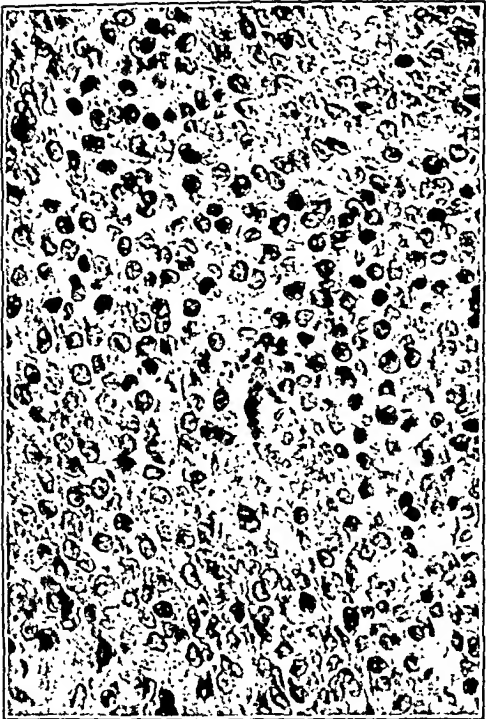


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PLATE 127

FIG. 12. Case II. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, $\times 300$.) Nuclei tend to be vesicular, vascular sinuses rare, no stroma. (*b*) (Neutral ethyl violet-orange G, $\times 850$.) Shows a single cell containing a ring of alpha-granules in the periphery of its cytoplasm.

FIGS. 13 and 14. Case III. Large, heavy-set man, with obesity and traces of acromegaly shown by the X-ray films.



a

12



b



13

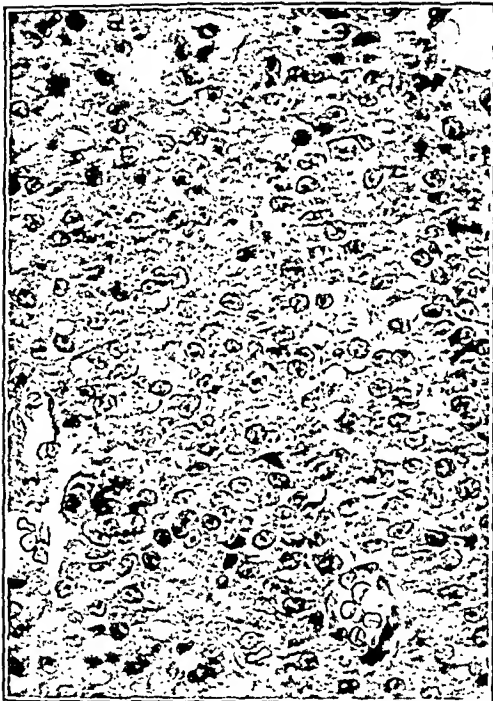


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PLATE 128

FIG. 15. Case III. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, $\times 300$.) Nuclei tend to be vesicular, sinuses rare, no stroma. (*b*) (Neutral ethyl violet-orange G, $\times 850$.) One cell is seen, in the center, with a rim of alpha-granules.

FIGS. 16 and 17. Case IV. Dyspituitarism with thickening and puffiness of the subcutaneous tissue of the face and with glycosuria.



a



b

15



16

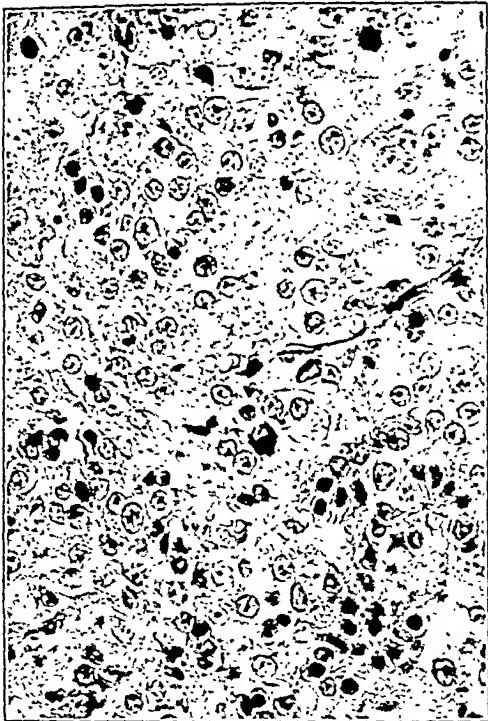


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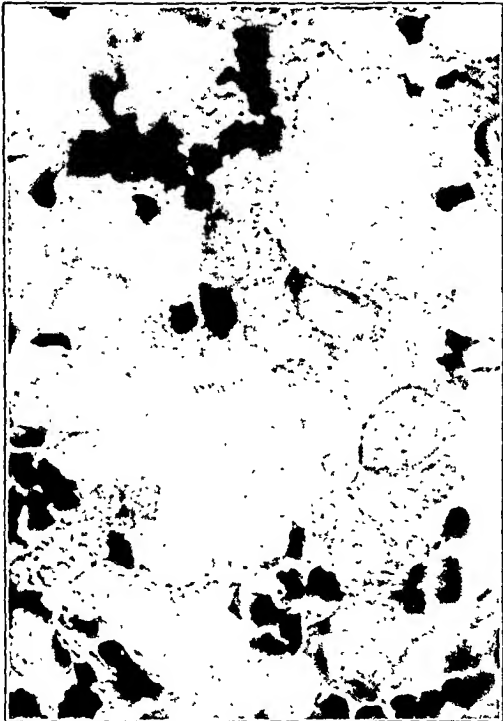
PLATE 129

FIG. 18. Case IV. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, $\times 300$.) Nuclei are mostly vesicular, the vascular sinuses are rare, there is no stroma. (*b*) (Neutral ethyl violet-orange G, $\times 850$.) Several cells are seen with a rim of alpha-granules.

FIG. 19. Hypophysis of new-born rat, showing the first appearance of alpha cells (neutral ethyl violet-orange G, $\times 850$).

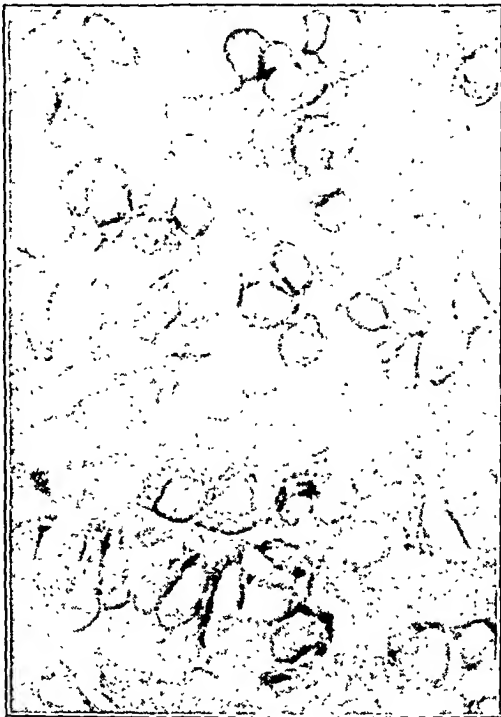


a



b

18



19

EXPERIMENTAL SUBCUTANEOUS RHEUMATIC NODULES*

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The subcutaneous rheumatic nodule is a localized area of inflammation, mostly proliferative in character, found in the loose connective tissue beneath the skin in a limited number of cases of acute rheumatic fever. Its structure is similar to that found in the Aschoff nodule in the heart or in rheumatic inflammation in other parts of the body, as in the joints, galea aponeurotica, diaphragm, blood vessels, etc.

Apparently the first written account of these subcutaneous nodules was given by Hillier¹ in 1868. In 1875 Meynet² pointed out that these nodular areas bore a direct relationship to rheumatic fever. The nodules, according to Angel Money,³ are less likely to occur in the extremely acute cases than in the chronic. Cheadle⁴ seldom found them in acute rheumatic fever except in childhood.

Hirschsprung⁵ in 1881 gave the first microscopic description of the nodules. He described them as consisting of connective tissue cells in different modifications. These cells varied in size and shape. Some were spindle-shaped and others were irregular. They contained one or more vesicular nuclei. These nuclei, he observed, were larger than those found in ordinary granulation tissue. He also noted that the ground substance between the cells consisted of a homogeneous material and that there was an increase in the number of blood vessels. In structure, the nodule resembled the tubercle, he thought, but did not have its typical appearance. He defined the nodule as being a localized area of inflammation with a tendency to undergo necrosis.

In 1881 Barlow and Warner⁶ described the reaction in these subcutaneous rheumatic nodules as being similar to the reaction in the valves in acute rheumatic endocarditis. The same observation has recently been made by Swift.⁷

The presence of a proliferative endarteritis in these nodules was observed by Cavafy⁸ in 1883. He noticed that the proliferating intima completely closed the vessel in some cases.

* Received for publication July 2, 1928.

Futcher⁹ in his microscopic description of the subcutaneous rheumatic nodule states that it consists of fibrous tissue in various stages of development. In some nodules he found the cellular element to be made up of small round cells, fibroblasts, and polymorphonuclear leucocytes. He also noted giant cells, some of which contained as many as twenty-six nuclei. All stages were present from the young cellular nodules to the older fibrous ones. Some nodules even became calcified.

The most complete microscopic description of the subcutaneous rheumatic nodules was made by Frank¹⁰ in 1912. He described them as being divided into a central and a peripheral portion. The center consisted of a homogeneous mass which stained intensely red with eosin, and by special staining he decided that this homogeneous material was fibrin. This fibrinous exudate extended out between the cells into the periphery. The periphery consisted of proliferative connective tissue which appeared partly as spindle cells and partly as epithelioid cells. Scattered among the proliferating cells a large number of leucocytes could often be seen. A variation in structure was noted in different nodules. This variation Frank considered to be due to different stages in the development of the same process, and he thought the primary reaction in the nodule was an exudation of polymorphonuclear leucocytes which was followed by a wandering in of round cells and by a proliferation of the surrounding connective tissue cells.

Swift⁷ described the subcutaneous nodules as being made up of a conglomerate number of smaller nodules and as being similar in structure to the nodules found in the heart and other parts of the body. He observed that the center consisted of necrotic material and a small amount of fibrin.

There seems to be a general agreement by observers who have studied the subcutaneous rheumatic nodules that they consist of proliferating connective tissue cells and a cellular exudate of lymphocytes, plasma cells, and polymorphonuclear leucocytes in varying numbers and that in the center of most of the nodules there is a greater or less amount of homogeneous substance consisting of necrotic material and fibrin. There is also agreement that anatomically and etiologically the subcutaneous nodule is similar to the nodule in the heart described by Aschoff,¹¹ Geipel,¹² Coombs,¹³ and others, and to the type of inflammation found in other parts of the

body in acute rheumatic fever. This type of inflammation has come to be considered by many observers a specific reaction to the rheumatic virus.

In this paper a microscopic study is made of human subcutaneous rheumatic nodules and of the nodules produced experimentally in the subcutaneous tissue in rabbits by injecting streptococci. The purpose is to compare the structure of a known rheumatic lesion with that of a lesion which has been produced experimentally to arrive at further conclusions concerning the causal relation of streptococci to rheumatic infections.

HUMAN SUBCUTANEOUS RHEUMATIC NODULES

The human subcutaneous rheumatic nodule which is studied in this paper was removed from the subcutaneous tissue of a patient having acute rheumatic fever. The nodule was found, upon microscopic examination of serial sections of the entire nodule, to be made up of many smaller nodular areas of inflammation. The reaction observed in this nodule was chiefly proliferative in character, but in many parts exudates of polymorphonuclear leucocytes, lymphocytes, and what appeared to be fibrin were noted (Fig. 1). The proliferative cells varied in size, shape and in staining qualities. Some of the nuclei were vesicular in character and some were hyperchromatic. The cytoplasm also varied in its degree of taking the basic stains. Many of these cells were multinucleated. Some of the nodular areas showed more or less necrosis in the centers. There was an increase in the number of blood vessels, and proliferative endarteritis was noticed in the smaller vessels. Morphologically the reaction observed in this nodule was similar to that described by Hirschsprung, Fletcher, Frank and others.

EXPERIMENTAL SUBCUTANEOUS RHEUMATIC NODULES

Nodules were produced in the subcutaneous tissues of rabbits by injecting different strains of streptococci in varying amounts and at different intervals. These nodules were removed and the microscopic structure was studied and compared with the structure of the human subcutaneous rheumatic nodule.

Five different strains of streptococci were used in the experiments. Two of these strains were isolated from the blood of patients having

acute rheumatic fever, one from the blood of a patient with subacute bacterial endocarditis, and two from pus from sinuses in cases of sinusitis. All of these organisms produced methemoglobin on the blood agar plate. Those strains isolated from the blood of patients with acute rheumatic or subacute bacterial endocarditis were less virulent to rabbits than the strains isolated from the sinuses.

Ten rabbits were injected intracutaneously and subcutaneously in many places with these organisms. Most of the animals had been previously injected with strains of streptococci intra-arterially through the left ventricle of the heart. Others had been previously injected subcutaneously in one area with a mixture of streptococci and agar. Just what part allergy may have played in the reaction in these experiments cannot be stated definitely.

The reaction found in the injected areas of the animals depended upon the virulence of the organism, the number of organisms injected, and the time of removal of the nodules after injection. The most virulent organisms tended to produce abscesses but in some cases typical proliferative nodules were produced. With the same organism the reaction varied according to the number of organisms injected. With a very few organisms proliferative nodules were produced, while with larger amounts abscesses resulted. With the same organism and approximately the same dose the nodules examined a day or two following the injection, as a rule, showed chiefly polymorphonuclear leucocytes, while in the nodules examined at from seven to fifteen days after the injection the chief reaction was proliferative in character. These findings are in agreement with Frank's conception of the pathogenesis of subcutaneous nodules, *i.e.*, the first reaction is an exudative one which is followed by a wandering in of lymphocytes and by a proliferation of the connective tissue cells. In all the rabbits injected some proliferative nodules were found, but such nodules were most frequent in the rabbits injected with small doses of the less virulent organisms. With the more virulent organisms there was a greater tendency for abscesses to develop.

In the proliferating nodules produced in rabbits many mononuclear and multinucleated cells with vesicular nuclei were observed (Figs. 2, 3 and 4). Some cells were large and irregular. Morphologically the cells found in these nodules appear similar to those in the human rheumatic subcutaneous nodules and in the human

Aschoff nodules in the heart. In some of these nodules, areas of slight necrosis were noticed. A varying number of polymorphonuclear leucocytes and lymphocytes were also present. There was an increase in the number of blood vessels and in some of these nodules there was an endarteritis.

It seems evident from these experiments that, by injecting streptococci into the subcutaneous tissues of rabbits, lesions can be produced which are morphologically similar to the nodules found in the subcutaneous tissue in cases of acute rheumatic fever. Since these experimental nodules occur obviously as a result of injecting streptococci, the probable conclusion is suggested that acute rheumatic fever and the type of inflammation associated with it are of streptococcic origin.

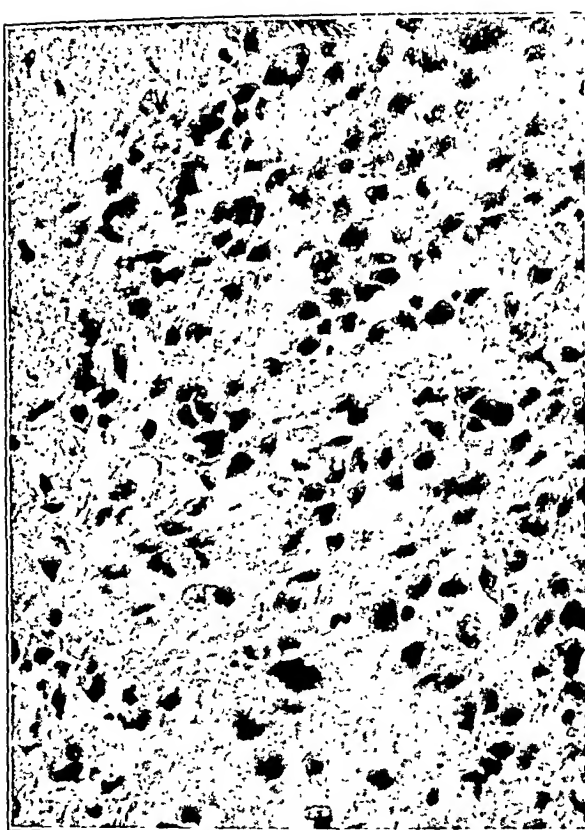
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DESCRIPTION OF PLATE

PLATE 130

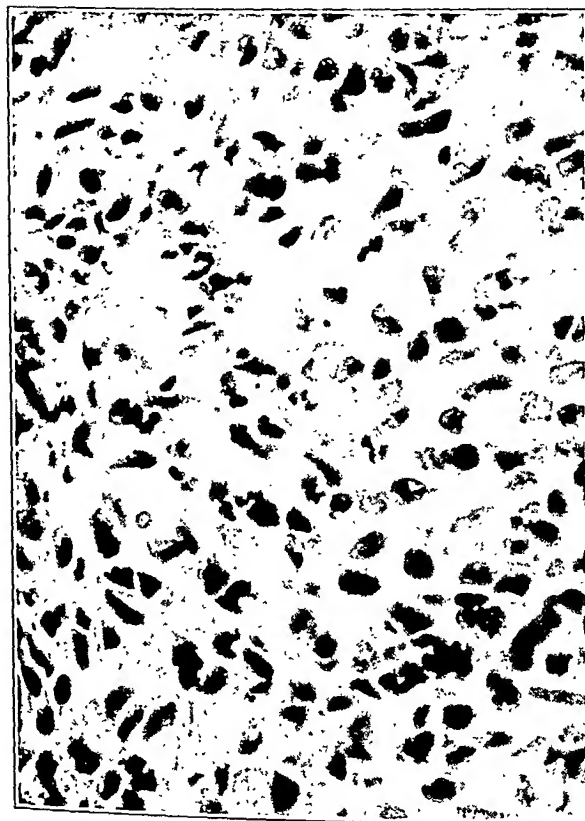
- FIG. 1. Human subcutaneous rheumatic nodule.
- FIG. 2. Experimental nodule in the subcutaneous tissue of a rabbit.
- FIG. 3. Experimental nodule in rabbit showing irregular proliferative cells.
- FIG. 4. Experimental nodule in rabbit showing mononuclear and multinucleated proliferative cells.



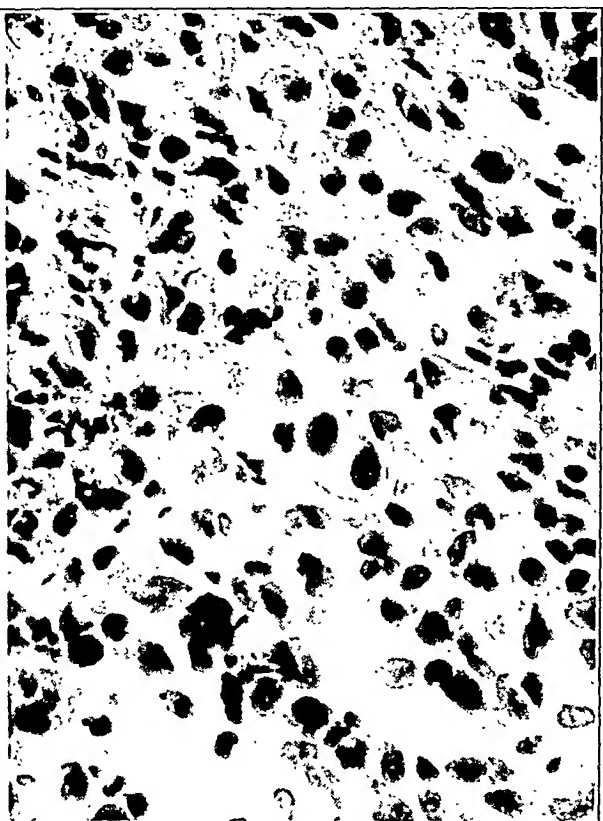
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4

EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED BY INTRARENAL TUBERCULIN REACTIONS *

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WITH THE TECHNICAL ASSISTANCE OF PAUL J. PATCHEN

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The tuberculin reaction, occurring when the specific protein of the tubercle bacillus comes into contact with the hypersensitive tissue of a tuberculous animal, illustrates several of the well known histological features of acute and chronic inflammation. Previous studies, of which this is an outgrowth, have shown that in a number of organs two broad phases of the reaction may conveniently be distinguished. These are (1) an acute exudative stage with degeneration or necrosis of local tissue, followed by (2) a stage of repair in which the products of the acute reaction are removed by absorption or proliferative organization of the exudate.

Both of these stages may be clearly seen in the familiar tuberculin skin reaction. They are still more conspicuous in the testis.¹ When a small amount of tuberculin is introduced into the testis of a tuberculous guinea pig, an initial acute exudative reaction occurs, with active hyperemia and escape of plasma and leucocytes into the interstitial tissue, and at the same time with severe injury to the cells of the seminiferous tubules, the spermatocytes being particularly affected. This reaction, which takes place within twenty-four hours, is followed by a period of absorption of the exudate and degenerated parenchymatous cells. At the end of a month the germinal cells, with the exception of the spermatogonia, are entirely absorbed, and the interstitial tissue is increased. The reaction is specific for the hypersensitive state, the introduction of tuberculin in the testis of the normal animal being without effect.

Tuberculin reactions of varying intensity may be produced in other organs. The results secured in the kidney have been found to

* Received for publication June 15, 1928.

† Aided by a grant from the National Tuberculosis Association.

be of particular interest, and seemed to us to warrant special investigation for a possible bearing upon the puzzling problem of spontaneous inflammation of the kidney in man.

That nephritis may at times occur as a manifestation of an allergic reaction has been many times suggested. This possible etiology has been particularly stressed by Longcope,² and supported by his observations on the effect of intravenous injection of foreign proteins into animals sensitized to these proteins. Changes developing in the kidneys resembled the lesions of nephritis in man.³

Rist and Leon-Kindberg⁴ noted an allergic response in the kidneys of tuberculous dogs after intracardiac injection of tubercle bacilli. Hemorrhage and diffuse degeneration of the renal epithelium occurred. Ophüls⁵ has advanced the view that allergic reactions may be at the basis of some of the glomerular forms of nephritis observed in man. Several investigators, including Ophüls, have stressed the possibility that an allergic element may be present in the inflammatory response of the glomeruli to the nephritis associated with subacute bacterial endocarditis. Thus an allergic ground for both degenerative and inflammatory lesions of the kidney has been suggested.

EXPERIMENTAL

Impressed by the severity of injury produced by tuberculin in the testis of a sensitive animal, we have attempted to produce analogous intrarenal tuberculin reactions. The first trial, injection of tuberculin preparations directly into the body of the kidney in guinea pigs, in a manner similar to that employed for testis inoculations, failed to yield results of significance. The attempt was then made to localize an inflammatory reaction to the glomeruli by arterial injection. Dogs, first selected for the purpose, proved to be unsuitable, because not sufficiently sensitive to tuberculin. Swine, which are sensitive to tuberculin following the artificial production of a mild tuberculosis, were next chosen. In the first experiments various preparations of tuberculin, and later dead tubercle bacilli, were injected with a 10 cc. glass syringe into the exposed renal artery under ether anesthesia. This procedure resulted in the production of scattered glomerular lesions of an early acute and later proliferative type.

In order to secure a more diffuse type of lesion a method similar to that recently reported by Miller and Apfelbach⁶ was used.

These investigators perfused the kidneys of dogs with a suspension of carbon particles in the effort to produce glomerular obstruction, allowing sufficient time for the normal cycle of variation in the circulation to different parts to occur. In this way the majority of the glomeruli were reached.

The apparatus used in the experiments here reported is shown in Fig. 1. The tuberculin preparation employed was a protein, highly active in the tuberculin test, isolated from tuberculin by Dr. Seibert in a previous investigation.⁷ This was dissolved in weak sodium hydrate and reprecipitated in a finely flocculent suspension by the addition of a few drops of N/10 hydrochloric acid. The suspension, which was faintly acid, was then added to a solution containing 1 per cent sodium citrate and 0.5 per cent sodium chloride. One hundred cc. of this preparation, containing 35-100 mg. of tuberculin protein, was introduced in the apparatus as shown in the figure, and pumped into the isolated renal artery of the experimental animal under ether anesthesia, the kidney being exposed extra-peritoneally by lumbar incision. By the use of the manometer shown, and observation of the point at which blood regurgitated through the needle into the glass syringe, it was possible to carry out the perfusion at a pressure 20-30 mm. Hg. above the blood pressure in the renal artery.

Six swine, two months old, were used in the final experiment here reported. Three of these were normal controls. Two had a localized lymph nodal tuberculosis produced by inoculation with 10 mg. of human type tubercle bacilli, and one a plastic peritoneal tuberculosis from intraperitoneal injection. Almost constantly the perfusion produced symptoms of shock in the tuberculous animals, while the normal pigs were unaffected. As a rule the left kidney was perfused first and the right kidney some weeks subsequently. In one instance a second perfusion of one kidney was made. The experiment was of a years duration. The anatomical effect of the injection was determined by biopsy, nephrectomy, or killing of the animal. The course of functional change was followed by analyses of the blood and simple examination of the urine. The blood analyses were made after the animals had been eighteen to twenty hours without food. Where blood analysis and operation were to be carried out on the same day, the blood for the chemical study was withdrawn first. Exhaustive study of the urine was not made, as trial soon showed

that the blood chemistry was a much more significant index of the condition of the animal.

The following protocols summarize the experiment:

Pig No. I

Tuberculous Pig, Infected Subcutaneously on Oct. 20, 1926.

Dec. 7, 1926. Left kidney perfused with suspension of 40 mg. of tuberculin protein in 90 cc. salt-citrate solution.

Dec. 21, 1926. Right kidney perfused with suspension of 70 mg. of tuberculin protein in 100 cc. salt-citrate solution. Profound shock during operation.

March 13, 1927. Left kidney again perfused with suspension of 70 mg. of tuberculin protein in 100 cc. salt-citrate solution. Marked asthma one hour after operation. Pig drowsy and inactive throughout next day.

July 7, 1927. Right kidney removed by operation. Found to be simply a small hydronephrotic sac. No pulsation in renal artery.

Dec. 2, 1927. Pig killed with ether.

Description of Kidney (left); Gross: There is a moderate induration of the perirenal tissue. The capsule strips with some difficulty leaving a slightly pitted surface. The cortex is a little irregular, with a few depressed scars.

Microscopic: The majority of the glomeruli are not much changed. A moderate number of glomeruli show a small amount of hyaline scarring and mononuclear infiltration at the point of entrance and exit of the glomerular vessels. A few glomeruli are completely fibrosed, with atrophy of the corresponding tubules. In general the tubules are normal. The blood vessels are normal.

Blood Nitrogen

	12/7/26	12/21/26	1/16/27	3/13/27	3/28/27	6/4/27	7/7/27	9/5/27
Urea N, mg. per 100 cc. .	13.3	14.0	14.0	14.0	35.0	19.3	14.0	18.2
Total non-protein N, mg. per 100 cc.	40.0	30.0	41.7	35.0	62.4	45.5	37.6	41.7
Creatinine, mg. per 100 cc.	1.3	1.2	1.5	1.6	2.1	1.7	1.9	2.0

Pig No. II

Non-Tuberculous Pig; Control to No. I.

Nov. 24, 1926. Left kidney perfused with suspension of 40 mg. of tuberculin protein in 90 cc. salt-citrate solution.

Dec. 8, 1926. Right kidney perfused with suspension of 100 mg. of tuberculin protein in 100 cc. salt-citrate solution.

July 7, 1927. Right kidney removed. Found to be simply a small hydro-nephrotic sac.

Nov. 18, 1927. Pig killed with ether.

Description of Kidney (left); Gross: The kidney shows very little change from the normal. There is no induration of the perirenal tissue. The capsule strips easily, leaving a smooth, normal surface. The cortex is regular and the markings normal.

Microscopic: There is practically no change from normal. Rarely a small scar with a slight amount of mononuclear cellular infiltration is found.

Blood Nitrogen

	11/24/26	12/8/26	1/23/27	6/4/27	7/8/27	9/5/27
Urea N, mg. per 100 cc. . .	13.0	15.4	14.0	20.2	11.2	11.2
Total non-protein N, mg. per 100 cc.	30.0	41.6	37.0	41.7	35.5	38.5
Creatinine, mg. per 100 cc.	1.2	1.4	1.5	1.8	1.9	1.8

FIG No. III

Tuberculous Pig, Infected Intraperitoneally on Oct. 19, 1926.

Dec. 10, 1926. Left kidney perfused with 30 mg. of tuberculin protein suspended in 90 cc. salt-citrate solution.

Feb. 11, 1927. Right kidney perfused with 65 mg. of tuberculin protein suspended in 90 cc. salt-citrate solution.

Feb. 14, 1927. Pig killed with ether. Pig found to have a slight fibroplastic peritoneal tuberculosis with adhesions between loops of bowel and moderate tuberculous mesenteric lymphadenitis.

Description of Left Kidney; Gross: The kidney is smaller than the right. The perirenal tissue is indurated. The capsule strips with difficulty, leaving a slightly pitted surface. On section the cortex is found to be irregular in thickness, showing numerous areas of atrophy.

Microscopic: About 50 per cent of the glomeruli show change, consisting usually of an epithelial proliferation within the tuft. Frequently the damage is confined to single loop of the tuft. Such loops are often hyalinized and show a few polymorphonuclear leucocytes and pycnotic tissue nuclei. Epithelial organization of exudate

in the capsular space is occasionally seen. A small number of glomeruli are completely destroyed, being replaced by proliferating epithelium. No glomeruli are fibrotic. The tubules are atrophic in the neighborhood of the most severely damaged glomeruli. A few casts are present. The blood vessels are normal.

Description of Right Kidney; Gross: The kidney is swollen, mottled, and flecked with red dots of pin-point size. The capsule strips easily.

Microscopic: The majority of the glomeruli are changed. Many show a fibrinous exudate in the capsular space, in which polymorphonuclear leucocytes and large mononuclears are present. In nearly all glomeruli there is a marked proliferation of the capillary endothelial cells. Hyaline casts, often containing polymorphonuclear leucocytes, are abundant. Tubules not containing protein are collapsed. No vascular injury is seen, but there are a few small, sub-capsular wedge-shaped infarcts.

Blood Nitrogen

	12/10/26	2/11/27*	2/14/27
Urea N, mg. per 100 cc.	10.5	18.2	34.3
Total non-protein N, mg. per 100 cc.	33.3	40.0	62.5
Creatinine, mg. per 100 cc.	1.3	1.6	2.0

* Pig had had access to some food within 12 hours preceding analysis.

PIG No. IV

Non-Tuberculous Pig, Control to No. III.

Dec. 14, 1926. Left kidney perfused with a suspension of 100 mg. of tuberculin protein in 100 cc. of salt-citrate solution.

Feb. 27, 1927. Right kidney perfused with 70 mg. of tuberculin protein in 100 cc. salt-citrate solution. The animal developed marked shock in the course of the operation, with much depression of the heart beat and respiration, but recovered in 20 minutes.

Mar. 2, 1927. Pig killed with ether.

Description of Left Kidney; Gross: There is no significant change except for some induration of the perirenal tissue.

Microscopic: Except for a few minute scars there are no changes. The glomeruli and tubules are normal. No changes are seen in the vascular system.

Description of Right Kidney; Gross: The kidney is profoundly changed. The perirenal tissue is edematous. The kidney is swollen. The capsule strips easily, leaving a mottled surface in which depressed white spots 2-3 mm. in diameter stand out sharply against a red background. There are some regions of superficial infarction. The cortical markings are in general obscured. The mucosa of the pelvis is swollen and hyperemic.

Microscopic: The glomeruli in all sections are much changed. Endothelial proliferation within the tuft is pronounced. Exudation of fibrin and leucocytes is seen in the capsular space in many instances. Practically all of the convoluted tubules contain hyaline casts. There are numerous small wedge-shaped regions just beneath the capsule in which both glomeruli and tubules are in a state of coagulation necrosis. Large vessels containing thrombi are not found, but some thrombi are seen in the venules.

Blood Nitrogen

	12/14/26	2/27/27	3/2/27
Urea N, mg. per 100 cc.	12.6	9.8	9.8
Total non-protein N, mg. per 100 cc.	31.2	25.0	27.8
Creatinine, mg. per 100 cc.	1.3	1.3	1.5

FIG No. V

Tuberculous Pig, Infected Subcutaneously, on Oct. 19, 1926.

Jan. 16, 1927. Left kidney perfused with a suspension of 100 mg. of tuberculin protein in 100 cc. salt-citrate solution. Pig had asthma for one hour after operation, but was in good condition next day.

April 1, 1927. Right kidney perfused with a suspension of 50 mg. of tuberculin protein in salt-citrate solution. In the course of the operation the pig developed profound asthma, so that it was necessary to stop the perfusion before the intended amount was injected.

April 5, 1927. The wound over the right kidney was reopened. The kidney was found to be pale but flecked with bright red spots. A piece was removed for examination.

Microscopic Examination: The glomeruli are enlarged, and the capsular space is distended. There is, however, no exudate of fibrin or leucocytes within the capsular space. Many of the cells of the tuft (epithelial?) show marked vacuolization. There is some proliferation of the capillary endothelium, and a good many polymorphonuclear leucocytes are present within the loops of the tufts. Many of the convoluted tubules are much distended and contain dense

hyaline or leucocytic casts. In many regions there is much diffuse leucocytic infiltration of the interstitial tissue.

Jan. 19, 1928. Pig killed with ether.

Description of Left Kidney; Gross: The perirenal fat is more densely adherent to the kidney capsule than normally. The renal capsule strips with great difficulty leaving a surface showing many minute depressions. The cortex is slightly decreased in thickness, and the cortical markings are somewhat obscured.

Microscopic: Except for small subcapsular scars there are no conspicuous changes. The glomerular tufts and capsules are for the most part normal, although occasionally there is some thickening and slight hyalinization of the tissue at the point of vascular attachment. The tubules are in general quite normal, as are the vessels and interstitial tissue.

Description of Right Kidney; Gross: Resembles the left, except that the capsule strips more readily, leaving a smoother surface, and that the cortex is wider.

Microscopic: Sections removed from all parts of the kidney, including the regions adjoining the biopsy site of April 5, show slight but definite change. The chief abnormality is a proliferation of elongated cells at the site of the vascular attachment of the tuft. The nuclei of the cells stain densely, and the cytoplasm is abundant and hyaline. Distal to this region the tuft is usually normal. Occasional patches of endothelial proliferation are seen, and such areas frequently contain pycnotic polymorphonuclear leucocytes. A very few glomeruli are fibrosed. In general the tubules are normal.

Blood Nitrogen

	1/16/27	4/5/27	4/12/27*	6/4/27	9/5/27
Urea N, mg. per 100 cc.	10.5	25.2	30.1	16.0	15.4
Total non-protein N, mg. per 100 cc.	34.5	55.5	69.0	38.5	37.0
Creatinine, mg. per 100 cc.	1.3	1.6	1.6	1.6	1.6

* Following day of heavy meat diet.

FIG No. VI

Non-Tuberculous Pig, Control to No. V.

Jan. 23, 1927. Left kidney perfused with a suspension of 100 mg. of tuberculin protein in 100 cc. salt-citrate solution. Marked blanching of kidney. No symptoms of shock.

April 26, 1927. Right kidney injected with 40 mg. of tuberculin protein in 100 cc. salt-citrate solution.

April 30, 1927. The wound over the right kidney was reopened, and a piece of tissue removed for examination.

Microscopic: Except for the presence of a very few hyaline casts the renal tissue was normal. The inflammatory picture seen in the kidney of Pig No. V was entirely lacking.

Feb. 2, 1928. Pig killed with ether.

Description of Kidneys; Gross: The kidneys are unequal in size, the left being smaller. Otherwise they are quite similar. In each case the capsule strips easily, leaving a smooth surface. The smaller kidney shows no scarring. The cortex is of normal width in each case, and the vessels are normal.

Microscopic: No significant changes are seen in either kidney. The right kidney shows a few glomeruli with slight scarring at the point of vascular attachment, similar to that seen in the right kidney of Pig No. V, but on a less extensive scale. A moderate number of the tubules in association with such glomeruli contain hyaline casts. Otherwise the tubules are normal.

Blood Nitrogen

	1/23/27	4/26/27	4/30/27	5/4/27*	6/4/27	9/5/27
Urea N, mg. per 100 cc. . .	14.7	13.3	14.0	16.1	17.9	11.9
Total non-protein N, mg. per 100 cc.	34.5	33.3	37.0	38.5	47.5	35.0
Creatinine, mg. per 100 cc.	1.5	1.6	1.6	1.8	1.8	1.8

* Following day of heavy meat diet.

DISCUSSION OF PROTOCOLS FOR PIGS I AND II

In this experiment no view of the kidney was obtained in the acute stage of the reaction, or the period of recovery. Accordingly the only information available on the immediate or early effect of the tuberculin injections is the record of the state of the animals

at the time of the injection and the condition of the blood subsequent to perfusion of the second kidney.

It is significant that no immediate reaction followed the injections of tuberculin protein into the normal pig, while in the case of two of the injections in the tuberculous pig general symptoms referable to the tuberculin perfusion occurred. The first injection was perhaps too small to bring them out. We originally intended to give 100 mg. of the protein on the perfusion of the second kidney, but the animal was in a state of such serious depression after the introduction of 70 mg. that it was necessary to stop the injection. This immediate or early reaction to tuberculin, in contradistinction to the late, well recognized, typical "tuberculin reaction," has been repeatedly observed by others, and is considered by Zinsser⁸ to be a true anaphylactic response. It is manifested on intradermic injection within twenty minutes, by a reddening which soon passes away, while the true tuberculin reaction in the skin develops only after some hours and persists for a day or more.

The blood chemistry yielded information of great value on the state of renal excretion in the course of the experiment. At no time was there an appreciable retention of nitrogen in the blood of the non-tuberculous animal. In the tuberculous pig, on the other hand, a definite increase in the urea and total non-protein nitrogen of the blood occurred after the second injection of the second kidney. The first injection had apparently not been effective; the injection, as we have noted, was relatively small and had not produced a general reaction. From the results of later experiments in which biopsies were made in the acute stage of the reaction (Pigs V and VI) or the animals were killed two months after the injection (Pigs III and IV), we have reason to believe that on March 28, when the blood nitrogen of Pig No. I was high, the left kidney was in a state of acute reaction, or just recovering from it, while the right kidney was in a state of repair after the operation of December 21 (or out of commission entirely, as suggested by the postmortem finding). The kidneys of the non-tuberculous pig, on the other hand, as we may assume from later experiments, were at no time inflamed, and excretion proceeded normally.

The total destruction of the right kidney in each animal was surprising, and probably without specific significance. Vascular occlusion apparently developed in both the tuberculous and non-tuber-

culous pigs, an accident which did not occur in any of the other four pigs of this series or in seven other swine used in preliminary experiments where injection by syringe was practised.

The relative insignificance of the renal lesions noted at the post-mortem examination of these animals approximately a year after the first injections, is of the utmost importance. Without the more carefully controlled experiment on Pigs No. V and VI its meaning would not be clear. In the light of the later experiment, however, we have reason to believe that in Pig No. I an initial acute inflammation occurred, followed by such nearly perfect repair that only minute scarring was left as evidence of the previous damage. The youth of the pigs at the beginning of the experiments may have been a factor in this recovery. In the period while the pigs grew two or three hundred per cent in size, the glomeruli developed correspondingly.

DISCUSSION OF PROTOCOLS FOR PIGS III AND IV

In this experiment the necropsy afforded an examination of one kidney three days after the injection of tuberculin, and of the other kidney two months after its corresponding treatment.

In the tuberculous animal both left and right kidneys disclosed a state of inflammation. In the right kidney, which had been perfused with tuberculin protein three days before the pig was killed, and one hundred and sixteen days after the animal had been made tuberculous by intraperitoneal injection with tubercle bacilli, acute exudative and early proliferative glomerular reactions were seen. The exudative phase is illustrated in Fig. 2. In all respects it was similar in type to that occurring in tuberculin reactions in the skin or testis. Its wide distribution in this case justified the designation of the lesion produced as an acute glomerulonephritis.

The left kidney, injected sixty-five days before the pig was killed, and fifty-two days after the pig had been made tuberculous, was found at necropsy to be in the stage of repair after an acute inflammation. The essential lesions were glomerular in location and proliferative in character. Both tuft and capsule took part in the latter process. Epithelial and endothelial organization of exudate, the remains of which could still be seen, was evident. Fig. 3 shows an epithelial crescent, and Figs. 4, 5, and 6 proliferations of tuft or

capsule. Fig. 6 illustrates the atrophy of those tubules dependent for their nourishment on the glomeruli involved.

Many of the glomerular lesions in this case were indistinguishable from those commonly described in subacute glomerulonephritis. The involvement was not quite so extensive, however, as that considered pathognomonic for this condition. As, moreover, the term subacute glomerulonephritis is usually reserved for a progressive lesion, while the lesion in this case was reparative, and probably presaged healing (judging from the experience of Pig No. V), it is perhaps better to designate the lesion in Pig No. III simply as a proliferative glomerulonephritis.

In general there was a good deal of similarity in the glomerular lesions in this animal to those described and illustrated by Bell, Clawson and Hartzell⁹ after intravenous injection of streptococci into monkeys, and by Duval and Hibbard¹⁰ following injection of streptococcus toxin into rabbits. It is noteworthy that the first-named series of investigators made repeated injections of streptococci, and that in the later injections a systemic reaction occurred. It seems probable to us that a tissue sensitization to the injected substance may have played a part in their experiments as well as in ours.

The blood nitrogen in the case of Pig No. III showed a noteworthy rise following the injection of the second kidney. It may be assumed from the normal figure obtained for the blood taken before the operation, that as long as one kidney was uninjured, no retention occurred. All the experience of this series has supported this view.

The left kidney of the control, non-tuberculous pig (No. IV), which had received an even larger dose of the tuberculin protein than No. III, was found seventy-eight days after the perfusion to be practically normal. Obviously it had not passed through the stage of acute inflammation seen in Pig No. III.

The right kidney, on the other hand, was found profoundly injured three days after its injection. It will be noted that this pig had suffered from severe shock at the time of the operation, of that character which we had previously interpreted as anaphylactic. By analogous reasoning it might be assumed that the anatomic changes found were of the same nature, *i e.*, that the marked injury of the kidney was virtually an Arthus phenomenon, following sensitization by the first injection seventy-five days before. This is the best

explanation we have to offer. Against it is the fact that only this once in all the series did this picture occur. Vascular injury was certainly responsible for part of the process but a good deal of it seemed truly inflammatory in character.

In this pig, analysis of the blood at no time showed nitrogen retention. This is to be associated with the fact that, unlike the situation in Pig No. III, one kidney was intact throughout the experiment, as proved by postmortem examination.

DISCUSSION OF PROTOCOLS FOR PIGS V AND VI

In this experiment a biopsy permitted a view of the kidneys of the two animals four days after the injection of tuberculin, and the postmortem examination nine months later afforded a comparison with the biopsy specimen.

In this case again it is noteworthy that the tuberculous pig suffered from asthma and shock at the time of each operation, while no such effect was observed in the non-tuberculous pig. The biopsy specimen from the tuberculous pig disclosed an acute nephritis, not as definitely glomerular in character as that seen in Pig No. III, but fully as severe and likewise as diffuse (Figs. 7 and 8). The biopsy specimen from the non-tuberculous pig, which had received the same treatment with tuberculin, showed no inflammatory reaction whatsoever.

As the protocols show, the kidneys of the tuberculous as well as the non-tuberculous animal one year after the first injection and nine months after the second, disclosed only minimal change from the normal. The histological picture at the end of the experiment was quite the same as that seen in Pig No. I. Fig. 9 illustrates the slight amount of persisting change in the tuberculous pig. Nevertheless we know that this kidney had at one time been in a state of diffuse, violent acute inflammation, and while no biopsy had been performed on the other kidney, there is every reason to believe that it was in the same state of acute inflammation following its injection.

The blood chemistry paralleled the anatomic injury. Following the tuberculin reaction in the second kidney, a definite retention of nitrogen, not present after the injection of the one kidney only, developed. This was accentuated by placing the animal on a heavy meat diet. Later, complete restoration of function occurred, as

shown by a return of the non-protein nitrogen of the blood to a normal figure. This is presumably to be correlated with the morphological recovery observed. In the non-tuberculous animal, in which the biopsy after tuberculin injection showed no appreciable change from the normal, the blood nitrogen remained normal throughout the course of the experiment.

SUMMARY AND CONCLUSIONS

The experiments described above demonstrate that, by the injection of tuberculin into the kidneys of swine made sensitive to this substance by the presence of a mild tuberculosis, it is possible to produce a diffuse inflammation of the kidneys that may properly be considered an acute glomerulonephritis. Moreover this effect is the result of a true tuberculin reaction, as it does not occur following the perfusion of the kidney of a non-tuberculous animal with tuberculin. We may therefore add the intrarenal tuberculin reaction to the intradermic, conjunctival, intratesticular and other anatomical varieties of tuberculin reaction previously described.

The immediate reaction in the kidney is followed by a subsidence of acute manifestations, and absorption and proliferative organization of the exudate. Many of the glomeruli in this stage show the epithelial and endothelial stimulation and growth seen in a late "acute proliferative glomerulonephritis," or in a subacute glomerulonephritis. True epithelial crescents are occasionally seen. Atrophy of the tubules associated with the more severely injured glomeruli occurs.

When the injury is bilateral, a moderate nitrogen retention develops. In the experiments recorded here, this was seen regularly in the tuberculous pigs which had been subjected to renal tuberculin perfusion, and never in the non-tuberculous animals similarly treated.

With the passage of several months following the intrarenal, or more specifically intraglomerular tuberculin reaction, almost complete restoration to an anatomical normal occurs, at least if very young pigs are used, as was the case in this experiment. As the animals grow and the kidneys develop to normal bulk, the glomeruli increase in size correspondingly. The only persisting signs of the former acute inflammation are small hyaline areas which occasion-

ally contain old pycnotic polymorphonuclear leucocytes. These are usually located at the point of vascular attachment of the tuft and capsule. Simultaneously with this anatomical healing, complete functional recovery also occurs, shown by a disappearance of nitrogen retention and return of the blood to normal.

The question naturally arises whether or not the artificial production of the nephritis here described has any relation to the spontaneous development of nephritis in man. It will be recalled that the majority of adult human beings in urban groups are sensitive to tuberculin, and therefore carry within themselves the potentiality for the type of injury herein recorded. It is conceivable that during the period of activity of a tuberculous focus, tubercle bacillus protein could be taken up, as by phagocytic cells, from the débris of the lesion and excite changes of an allergic nature at a distant point such as a glomerulus of a kidney. It is unlikely, however, that any such dosage as used in the experiments described could be so absorbed. It is possible, on the other hand, that occasional and scattered glomerular lesions could arise in this way. However, it must be remembered that the tuberculin reaction is only one of a type. A considerable number of analogous forms of sensitization exist. While such reactions are in general protective against the spread of the infection concerned, the act of protection itself may be destructive to the local tissue, and no initial distinction is made in the reaction to live bacteria or their dead specific protein. These facts, together with the experiments reported here, justify further work in this field.

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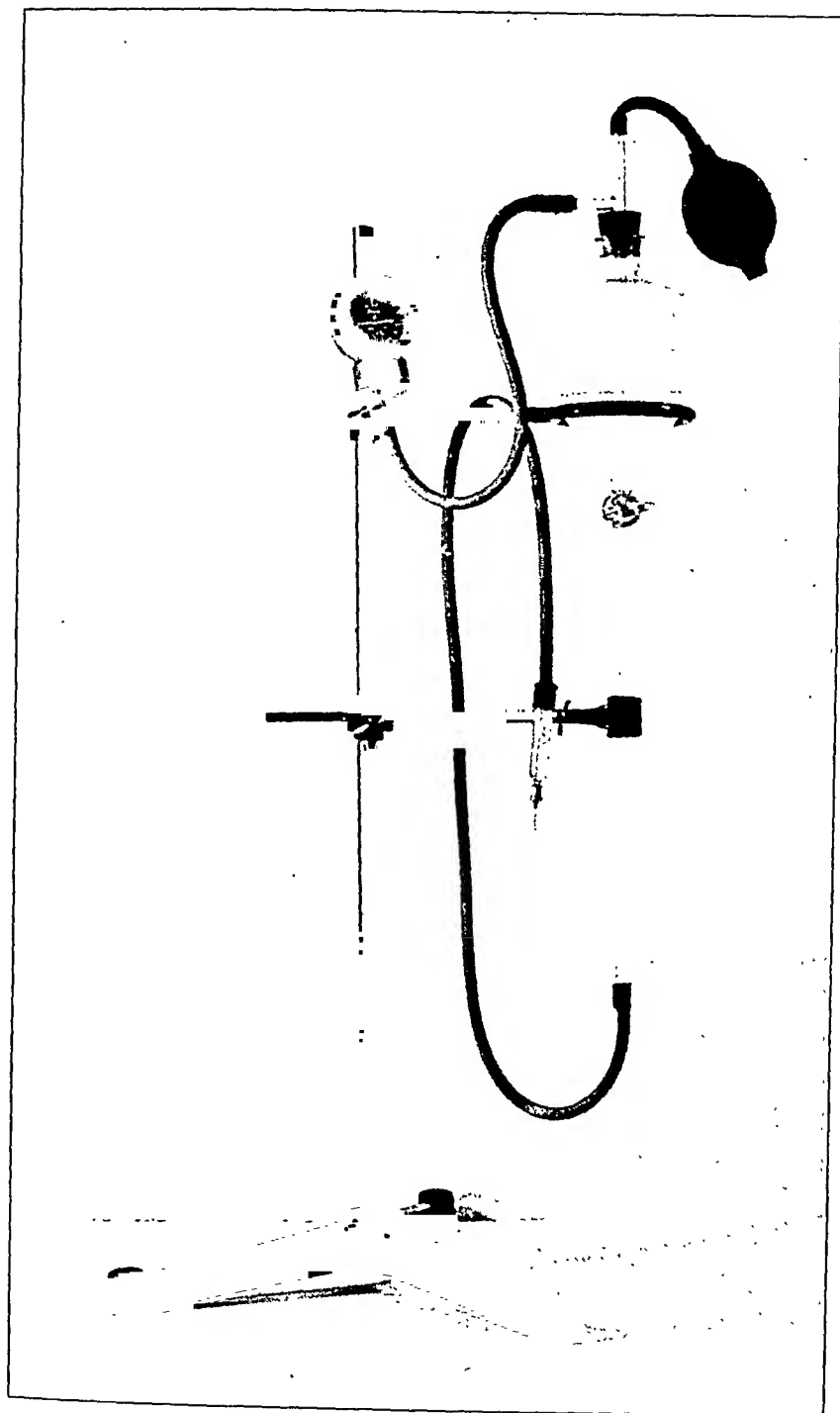
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DESCRIPTION OF PLATES

PLATE 131

FIG. 1. Apparatus used for tuberculin perfusions. The glass bulb contains a suspension of tuberculin protein.

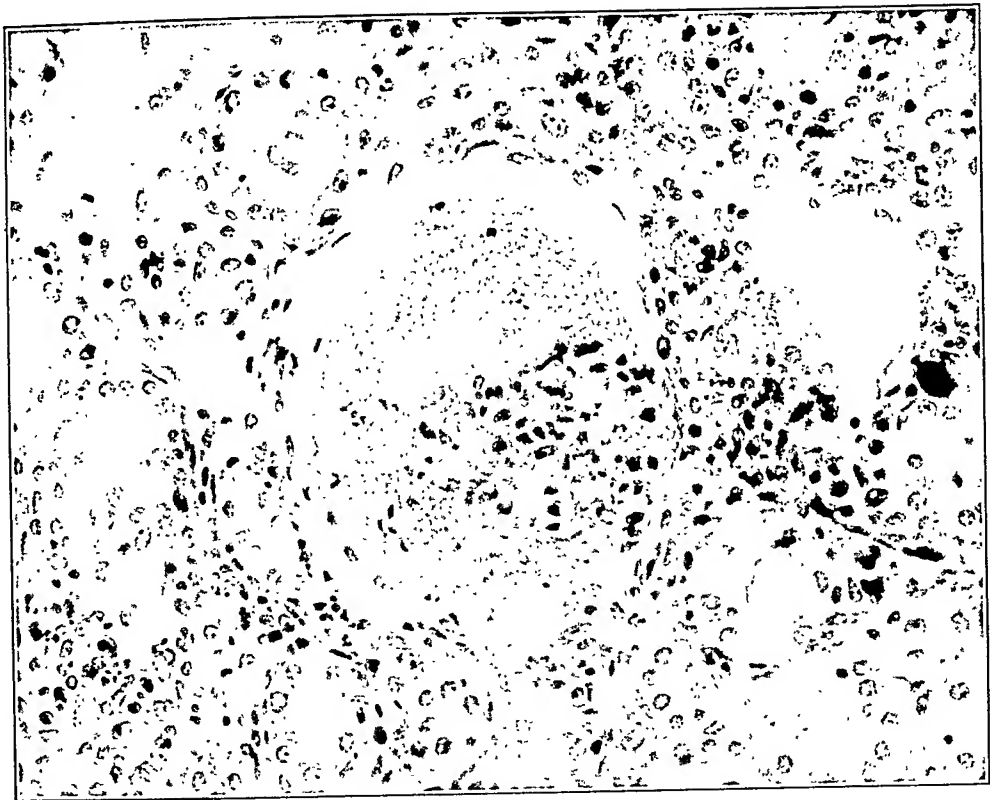


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PLATE 132

FIG. 2. Acute exudative (tuberculin) reaction in glomerulus. From Pig No. III (tuberculous pig) three days after injection of tuberculin protein into right renal artery. $\times 300$.

FIGS. 3. Proliferative lesions in glomeruli. From Pig No. III (tuberculous pig) sixty-five days after injection of tuberculin into left renal artery. $\times 200$.



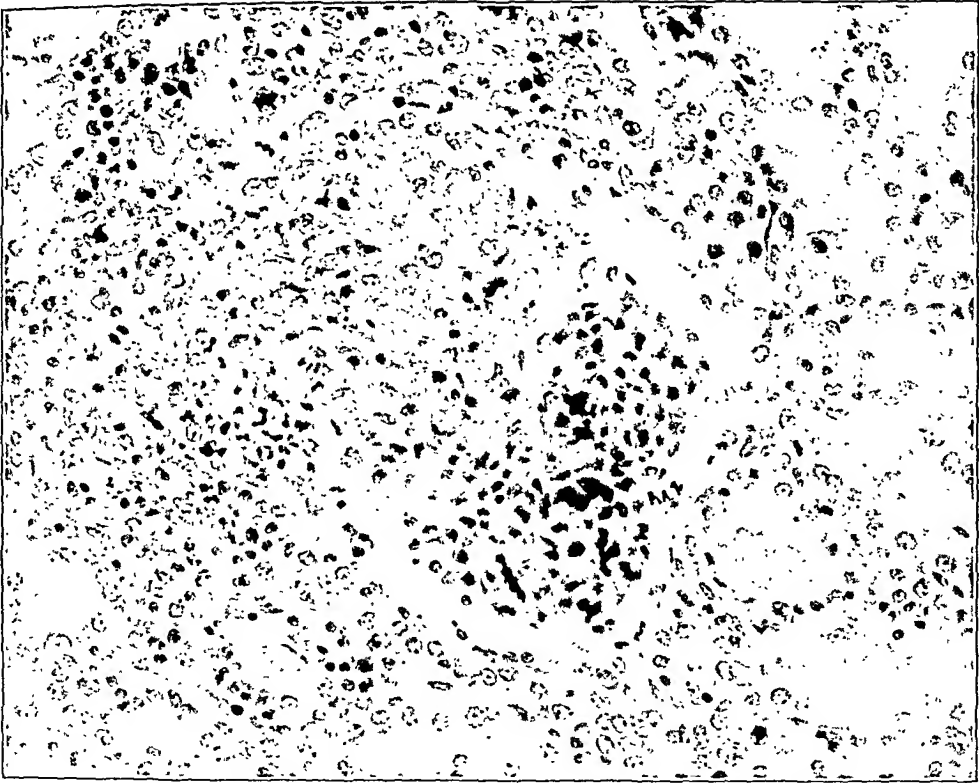
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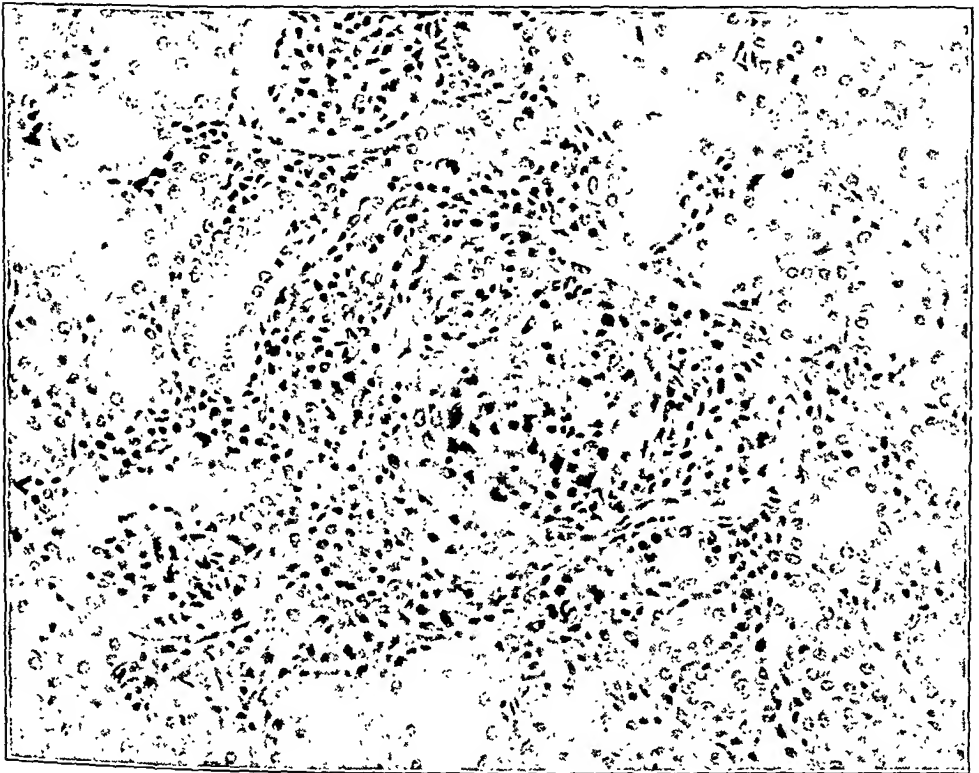
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PLATE 133

FIGS. 4 and 5. Proliferative lesions in glomeruli. From Pig No. III (tuberculous pig) sixty-five days after injection of tuberculin into left renal artery. Fig. 4, $\times 300$. Fig. 5, $\times 250$.



4



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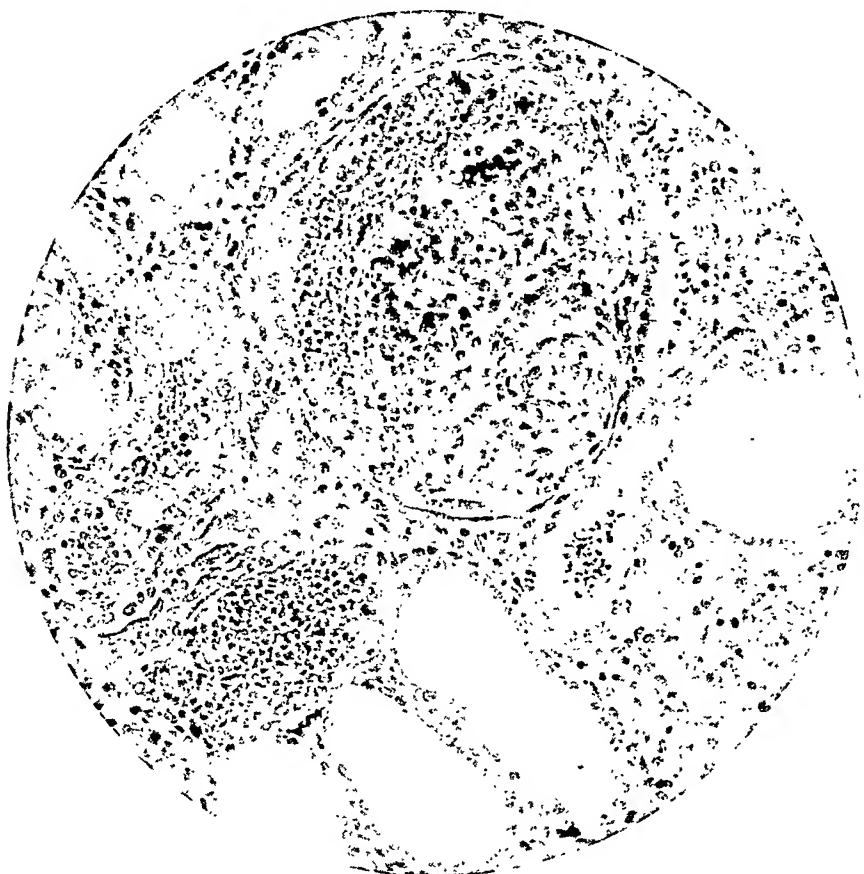
PLATE 134

FIG. 6. Same as Figs. 3, 4, and 5. $\times 170$.

FIG. 7. High power view of renal lesion in Pig No. V (tuberculous pig) four days after injection with tuberculin protein (biopsy). Note glomerular hemorrhage, increased cellularity of tuft, and blood and leucocytes in tubules. $\times 300$.



6

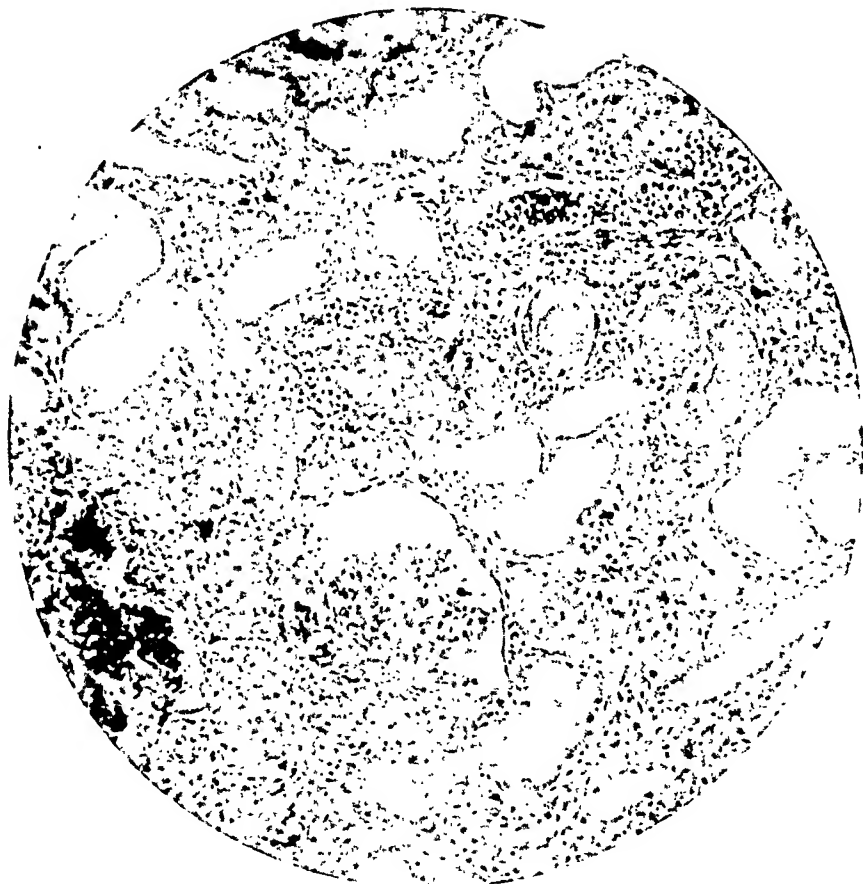


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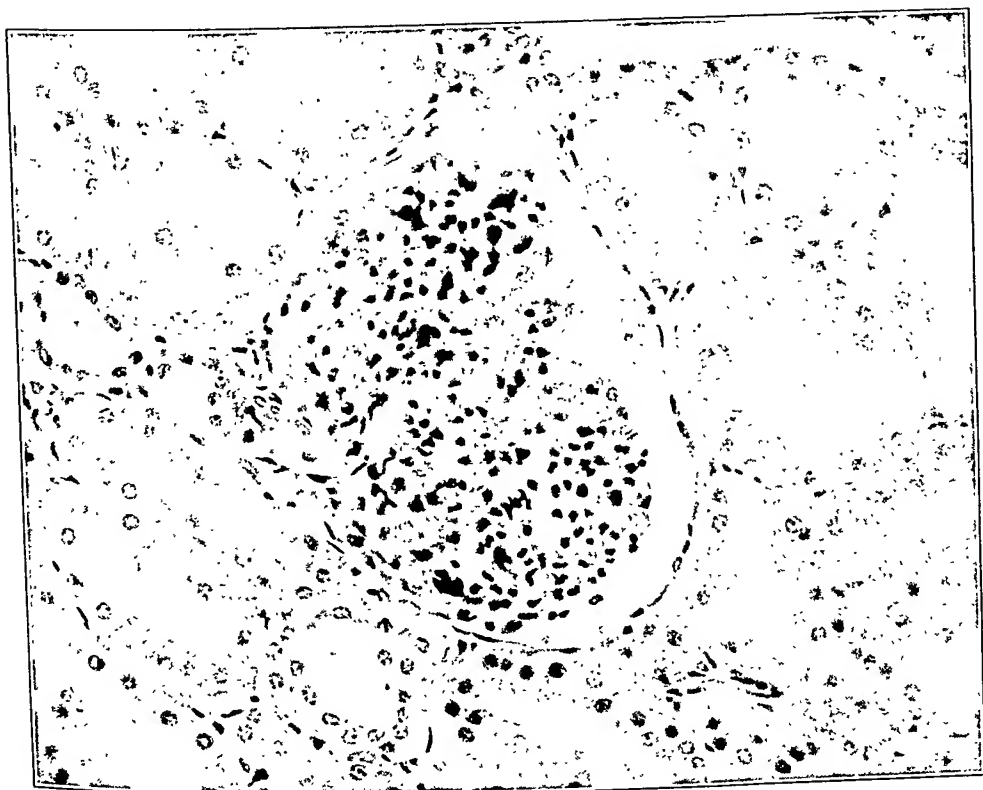
PLATE 135

FIG. 8. Same as Fig. 7. Note hyaline and leucocytic casts and distension of tubules. $\times 150$.

FIG. 9. Slight persisting change in glomerulus nine and one-half months after injection of tuberculin protein. From Pig No. V (tuberculous pig) and from the same region of the kidney as the biopsy specimen illustrated in Figs. 7 and 8. Note the small amount of hyaline scarring at the point of vascular attachment of the tuft. The rest of the glomerulus and the tuft are normal. $\times 300$.



8



9

THE PHAGOCYTIC ACTIVITY OF VASCULAR ENDOTHELIUM OF GRANULATION TISSUE *

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Much opposition has arisen to the view that the vascular endothelium may give rise to wandering phagocytic cells, and those sponsoring this opposition are inclined to the idea that the vascular endothelial cell is a highly differentiated element functioning only as a lining for the vessels. In an investigation including a study of the endothelium of the pulmonary capillaries Gardner and Smith¹ state: "Even after preliminary irritation with India ink these cells (the capillary endothelium) have failed to exhibit evidence of multiplication or of specific staining." Stilwell² ascribes to the endothelium a mere passive rôle in the handling of particulate matter. He thinks that carbon particles pass from the circulation through intact endothelium and are taken up by perivascular cells. Maximow³ admits that the Kupffer cells of the liver are phagocytic and are active in storing colloidal dyes but he differentiates sharply such "histiocytic littoral" phagocytes from the ordinary endothelium lining capillaries. Foot⁴ in a recent paper has abandoned his former opinion that the vascular endothelium is active in the production of phagocytes. He also apparently is inclined to the view that cells, such as the Kupffer cells of the liver, are anchored monocytes and not true endothelial cells. Sabin, Doan, and Cunningham⁵ by supravital staining distinguish two types of connective tissue phagocytes, the monocyte and the clasmatocyte, which is a wandering endothelial leukocyte.

In my initial phagocytic experiments with suspended carbon, I⁶ did not examine newly formed endothelium such as the vascular sprouts of granulation tissue. At this time, however, it was determined that stimulated endothelium, not only of the liver sinusoids but of the capillaries of other organs (heart), may ingest particles of carbon. I cannot subscribe to the expression of Stilwell that the incorporation of such particulate matter is passive. All carbon

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particles removed from the circulation do not pass passively from the endothelial-lined tube through cell interspaces, although some of them certainly do this, but a part of the microscopic granules are actively phagocytosed into the cytoplasm of the endothelial cells. The Kupffer cells of the liver are continuously called upon to remove particulate matter from the blood stream and have for the most part a more abundant cytoplasm than the endothelium of capillaries elsewhere, but structurally these active cells are not only continuous with the capillary endothelium of the vessels emerging from the periportal connective tissue of normal liver but in the repair of wounds of the liver these sinusoids are continuous with the capillaries of granulation tissue. It was, and is now, my opinion that the usual, flat, inactive endothelial cell, functioning as a vascular lining, is non-phagocytic; but under suitable stimulus it may enlarge, undergo mitosis and become actively phagocytic. That the chief function of the blood vascular endothelium is to afford a smooth vessel lining is perfectly obvious but there appears to be a basis for the opinion that the capillary endothelium of certain normal organs may ingest particulate matter and so remove it from the circulation. Likewise under influence of a sufficient stimulus the usual type of endothelium elsewhere may assume this phagocytic rôle.

METHODS AND RESULTS

The experiments were planned to determine the phagocytic possibilities of the endothelium of vascular sprouts in granulation tissue. The presumption was that some of the cells would become separated to give rise locally to free phagocytes provided the lining cells of the new vessels were found to be phagocytic. Proof of such local origin of free phagocytes was not included in the scope of the experiments. Carbon was used to test the phagocytic property of the cells. The granulation tissue was produced in some animals by the subcutaneous injection of large quantities of India ink and in others by the injection of suspended casein. Following the injection into the groin of 2 cc. of India ink (Higgins) the carbon tends at first to be encapsulated by the formation of vascularized connective tissue at its periphery. Some of the carbon at once passes to the regional lymph nodes where it marks some of the reticulo-endothelial cells.⁶ In an examination of many blocks of such encapsulated carbon it was only

in an occasional section that the endothelium of the new vessels was found to be marked with carbon. When such a vessel was located the lining endothelial cells usually contained much carbon (Fig. 1). Owing to this irregular distribution the inference was drawn that the new vessels come into contact with the carbon but the particles do not enter their lumina and are not taken up by the endothelium. Ink may have entered, through mechanical rupture, the lumina of the vessels in which the endothelium was found to contain ink particles. A wider distribution of ink in the vessels was obtained by introducing the ink directly into the vascular system.

Experiment A: Using a syringe fitted with a large needle, half-grown rats were injected subcutaneously in the groin and axilla with 1 cc. of a sterile thick suspension of casein. After nine to twenty-one days the animals were etherized and 1 cc. of India ink injected into the heart. After five minutes the rats were chloroformed and the carcasses placed at 37° C for thirty minutes. After this incubation the groin and axillary masses about the encapsulated casein were fixed in Zenker's fluid, embedded in paraffin and sectioned. The inflammatory reaction and the character of the granulation tissue varied with the time following the casein injection, but in all animals there were numerous newly formed vessels in the sections. Although the lungs, liver and spleen were a slate color, the capillaries about the encapsulated casein contained comparatively little ink. Ink within the endothelial cells was so scant that its presence there was questionable. Ink-containing phagocytes within the vessels were readily demonstrable. Later a rabbit, while being injected intravenously with India ink, died suddenly and was incubated for thirty minutes. As was the case with the rats, the endothelium was practically devoid of carbon.

Experiment B: Each of two young rabbits, weighing 675 gm. and 750 gm., received on June 1 six subcutaneous injections of casein. On June 25 each received an intravenous injection of 0.5 cc. India ink. On July 6 at 10 A.M. each received 1 cc. of India ink intravenously. Two hours later the animals were chloroformed and the lumps of encapsulated casein at once fixed in Zenker's fluid.

Although the liver sinusoids contain huge masses of agglutinated ink particles there is comparatively little ink in the casein nodules. The description and illustrations are of the two rabbits with double ink injection. Multiple microscopic nodules have formed about

small masses of casein. At the centers are casein particles encircled by a zone of polymorphonuclear leukocytes. The next zone consists of granulation tissue with a varying proportion of large mononuclear phagocytes, giant cells, lymphocytes, polymorphonuclears, new blood vessels, and connective tissue. About one per cent of the large mononuclear phagocytes contain carbon and more than one-half of the giant cells are marked with carbon. This carbon, and also the abundant carbon in the periportal connective tissue in the liver, is evidently from the first ink injection. Endothelial cells with ink particles are most abundant in the periphery of the granulation tissue abutting on muscle, adipose tissue, or loose connective tissue. Practically no free carbon is present in the lumina of the vessels but intravascular mononuclear phagocytes marked with ink occur. The capillaries within the dense leukocytic zone toward the casein contain carbon but in the sections they are collapsed and devoid of the red blood corpuscles which are so helpful in identification. It is therefore at the periphery of the casein nodules that discrete definite carbon particles are best seen within the cytoplasm of the endothelium of capillaries. Usually the vessels that show best are in longitudinal section and are sufficiently dilated to be cut through the walls on each side (Figs. 2 and 3). When the vessel wall in longitudinal section is not cut through it is often difficult to determine whether the carbon is intracellular or within the vessel lumen. To find a capillary cut across with carbon in the wall requires considerable search (Fig. 4). Taking into account the relatively small quantity of ink present in the nodules, it is obvious that it is only in an occasional capillary that the endothelial cells make contact with the carbon so as to permit phagocytosis.

DISCUSSION

Since these experiments show that vascular endothelium of granulation tissue may phagocyte carbon, just as the stimulated endothelium of various organs was found to do in earlier experiments, the blood vascular endothelium must be considered as a possible source of mononuclear phagocytes. Mallory⁷ was the first to recognize the endothelial origin of the mononuclear phagocyte of the blood and tissues. In a discussion of the histology of typhoid fever lesions that author⁸ stated that not only the vascular endothelium but also

the reticulo-endothelium of the lymphoid tissue gave origin to endothelial leukocytes. By marking the endothelium with carbon experimentally, I⁶ reached the conclusion that the mononuclear phagocytes were of endothelial origin. The deduction was based chiefly on the structural resemblance between carbon-containing endothelial cells and the free phagocytes with carbon. The reticulo-endothelium of lymphoid tissue, as well as the blood vascular endothelium, was found to phagocyte the carbon and at this time was stated to be a source of mononuclear phagocytes. There were not sufficient data to determine which of the two types of endothelial cells was the major source of these phagocytes. Sabin, Doan and Cunningham⁵ summarize as follows: "Thus we consider that the weight of evidence points to two separate strains of phagocytic cells of the connective tissue: clasmatocytes, which are of endothelial origin and come into the blood stream only occasionally and abnormally, and monocytes, which are a constant type of blood-cell, arising largely in the spleen, but also a specific cell of the diffuse connective tissues, where they both arise and function." My own work,^{9,10,11,12,13,14,15} convinces me that the conclusion reached by Sabin and her associates in regard to the clasmatocyte (hemendotheliocyte) is correct. By experimental procedure this cell may be made to enter the blood stream. Doubtless it usually arises from active endothelium such as the Kupffer cell, but capillary endothelium elsewhere (heart, granulation tissue) has the same potentiality. Also I agree with these investigators that there is a second type of phagocyte, the monocyte; but my experiments tend to show that this cell is derived from the reticular portion of lymphoid tissue wherever found. This lymphendotheliocyte is practically the sole mononuclear phagocyte of normal peripheral blood. It is also the usual mononuclear phagocyte of a variety of lesions. No conclusive work exists to show whether there is a relationship between the reticular and lymphocytic cells of lymphoid tissue. Once differentiated, I have found no evidence that the lymphocyte may enlarge to become a reticular phagocyte.

CONCLUSIONS

1. Carbon is present in the vessel walls of granulation tissue after India ink has been injected intravenously and also to some extent at the site of subcutaneous ink injections.

2. The presence of this particulate matter within the cytoplasm of the endothelial cells can be explained only as a process of active phagocytosis.

3. The phagocytic activity of these endothelial cells is identical with that of the Kupffer cells of the liver which have a comparable amount of cytoplasm.

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DESCRIPTION OF PLATE

PLATE 136

FIG. 1. Carbon in endothelial cells of a dilated vessel at site of subcutaneous ink injection. Section is tangential and vessel contained red corpuscles beyond portion in illustration.

FIG. 2. Capillary with phagocytized carbon from periphery of casein nodule of rabbit (Experiment B).

FIG. 3. Tangential section of vessel near periphery of a nodule (Experiment B). Heavy carbon masses in endothelial cells.

FIG. 4. Transverse section of capillary, well within a casein nodule (Experiment B).

NOTE: The structures were outlined with camera lucida. The drawings were made by Miss M. E. Bakehouse of the Department of Anatomy.



McJunkin

Phagocytic Activity of Vascular Endothelium

PRIMARY CARCINOMA OF THE LIVER: TWO CASES IN CATTLE *

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The spontaneous formation of tumor in the liver is not common. The organ suffers more often as a repository for metastasis from primary tumor elsewhere in the body.

According to Ewing, primary carcinomas of the liver constitute between 0.5 and 1.3 per cent of all epitheliomas in man. Authentic data concerning the incidence in the lower animals are very meager; Sticker's¹ figures are perhaps the most comprehensive. He reported the liver to be the site of primary origin in thirty-five of a total of 1206 primary carcinomas of the common domesticated animals. These thirty-five cases were distributed among the various species as follows: three in horses, four in oxen, five in sheep, twenty in dogs, two in cats, and one in a hog. In sixty-three cases of carcinoma and adenocarcinoma of the lower animals reviewed by Fadyean,² six cases of primary carcinoma of the liver are listed. These include three in dogs, two in sheep, and one in a cow. Hodgson³ described one case of primary carcinoma of a sheep's liver. The remainder of the abdominal viscera were not examined and Hodgson was in doubt as to whether the tumor was primary in the liver. However, in view of the extreme rarity of carcinomas in the gastro-intestinal tract of sheep, and the fact that most internal tumors of the sheep have been carcinomas of the liver, I believe that Hodgson's case was primary in this organ. Murray⁴ listed seven cases of malignant adenoma of the liver in a total of twenty-four malignant tumors in cattle. Primary carcinoma of the liver was not observed in twelve cases of malignant tumors in horses, and in eleven in cats. Murray did, however, find a "columnar cell carcinoma" in the liver of an old dog in a total of forty-nine malignant cases in dogs. In a case described by Ross⁵ a primary carcinoma of the liver, invasive in nature, was found in a dachshund. From Ross' description one is

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led to believe that his case represented a true carcinoma of the liver. Cohrs⁶ reported several cases of multiple neoplasm in dogs in which carcinoma of the liver was present and one case in a cat, associated with a cystadenoma of the gall ducts. Fox⁷ found an adenoma simplex of the liver of a woodchuck. Hutyra and Marek⁸ stated that primary carcinoma of the liver is rare in all the domestic animals but is most common in the dog. Kitt⁹ noted this in old dogs. Roussy and Wolf¹⁰ stated that the liver of cattle is frequently the site of adenomas, epitheliomas and sarcomas, and that they are usually associated with the presence of parasites (nematodes). Roussy and Wolf also mentioned that the parasitic livers of sheep not infrequently contain tumors similar to those found in cattle.

Siedamgrotzky¹¹ reported the case of a primary carcinoma of the liver in a hen with metastasis to the intestines. Joest and Ernesti¹² described two cases of primary carcinoma of the liver in a series of twenty carcinomas of birds; in one case the carcinoma was widely disseminated throughout the organ. Metastasis had not occurred. Pentimalli¹³ did not observe primary carcinoma of the liver in a series of eighteen chicken tumors. In my study of twenty-seven neoplasms in chickens I did not find evidence of primary carcinoma of the liver. I believe it is one of the rare tumors in chickens.

By far the most comprehensive description of primary carcinoma of the liver in cattle is that by Trotter.^{14, 15} He had the unusual opportunity to study 119 cases which were found in a total of 39,704 slaughtered cattle during one year at a Glasgow abattoir. Later he reported another case. Of the 120 cases all but two were in animals from the north or northwest of Ireland. The condition was more common in the older animals, "frequently associated with adenoma, chronic venous congestion, cirrhosis, distomatosis, cavernous angioma or, but most rarely, tuberculosis."

In one of Trotter's cases the affected liver weighed 36.3 kg. (80 pounds). Trotter gives a splendid picture of the metastatic possibilities of primary carcinoma of the liver and mentions that on several occasions emboli of tumor cells were found firmly attached within the lumen of one of the smaller branches of the portal vein. He observed that the thrombi were found more often in the lumen of the branches of the portal vein than in those of the hepatic artery or vein, and believed that the growing tumor cells cause so much pressure on the walls of the vessels as to invaginate the walls.

The tumor continues to expand and eventually the wall of the vessel ruptures, permitting the thrombus of tumor cells to project into the lumen of the vessel. Such a situation favors the dissemination of those tumor cells which become loosened from the parent mass, and as a consequence distant metastasis is possible. Trotter found instances of secondary foci in the lungs and in one case he found metastasis to the cortex of the kidney. Although the hepatic lymph nodes were often affected, metastasis occurred more frequently by way of the blood stream. Trotter also noted a "villous-like growth" in the lumen of the gall-bladder which had broken through the walls from its primary focus in the liver. In only one case was the peritoneum involved and in this case both the visceral and parietal surfaces were studded with secondary nodules of varying sizes. In this instance the hepatic, renal, and right internal iliac lymph nodes were also involved. Trotter observed a few cases in sheep.

In my series of eighty cases of neoplasms in cattle there were two of primary carcinoma of the liver. These are reported herewith.

REPORT OF CASES

CASE I. Material was received from a veterinarian in the federal meat inspection service, Denver, Colorado. The animal was a four-year-old Herford cow in apparent health and was slaughtered for food. Examination of the carcass, however, showed that the parietal surface of the liver presented several small nodular grayish tumors measuring from 0.5 to 2 cm. in diameter, which were grouped around a larger central mass measuring about 7 cm. in diameter. A few areas of necrosis and calcification were also observed. Because of these unusual lesions the veterinary inspector made a thorough examination of the head, lungs, kidneys, spleen, adrenals, gall-bladder, bones, lymph nodes, and intestines, but evidence of other tumors was not found.

Histologic Examination: This showed the normal structure of the liver to be interrupted by irregular collections of closely packed cells which stained strongly acidophilic. The larger areas of tumor cells were definitely separated from the tissue of the liver by broad bands of dense fibrous connective tissue. The smaller collections of cells did not have such barriers between them and the liver and seemed to be undergoing extension by infiltration into the remaining tissue of the liver (Fig. 1).

The parenchyma of the tumor consisted of large polyhedral cells resembling in many respects hepatic cells but lacking an orderly arrangement. The cytoplasm was minutely granular but a definite

cell wall could not be demonstrated. The nuclei were also large and in practically every instance a prominent nucleolus could be seen. Many of the nuclei appeared vesiculated and in the more immature forms they were strikingly hyperchromatic. Mitotic figures, while not abundant, were nevertheless frequently seen (Fig. 2).

Blood capillaries were present among the tumor cells, as were spaces suggestive of bile capillaries, but bile ducts could not be demonstrated. Sections stained by Best's carmine method revealed the presence of glycogen in the tumor cells.

The hepatic cells adjacent to the tumor were compressed and atrophic and were arranged in slender columns running parallel to the fibrous connective tissue which separated the neoplastic elements from those of the liver. The capsule of the organ was irregularly thickened and some of the subcapsular areas showed marked congestion of the blood capillaries. Extensive lymphocytic infiltration was a conspicuous feature of most of the fields (Fig. 1). The lymphocytes were distributed among the various collections of tumor cells and seemed to be most abundant in areas in which a reticular stroma was present. Cirrhosis was not observed. A diagnosis was made of primary carcinoma of the liver.

CASE II. A veterinarian in the federal meat inspection service also supplied the material for this case. The animal was an old-grade cow in very poor condition and the carcass was condemned on account of emaciation. At necropsy a large, flattened mass was found extending slightly above the parietal surface of the liver. The mass measured 20 by 12 cm.; it was firm, and of a dirty flesh color. On cross-section the mass was found to be rather sharply separated from the surrounding scant amount of liver tissue, which was greatly compressed by the expanding tumor (Fig. 3). There were many diffuse brownish areas throughout the mass and a few cystic cavities were apparent. Metastatic deposits were not found on careful examination of the carcass.

Histologic Examination: The substance of the liver in this case could barely be detected, the tissue consisting mostly of wide areas of large, strongly acidophilic cells not unlike those described in Case I. The type cell was polyhedral in contour with a finely granular cytoplasm and a large nucleus containing a prominent nucleolus. Most of the nuclei contained many chromatin granules and a few were vesiculated (Fig. 4). A few cells undergoing mitotic division were seen. The cells were without apparent arrangement, being disposed in a haphazard manner with an occasional suggestion of small

groups or clumps. Blood capillaries were generously distributed among the tumor cells, but bile ducts were not demonstrable.

The tumor was cut at irregular intervals by wide strands of fibrous tissue and in the few areas in which hepatic tissue remained an occasional large pink homogeneous cyst was found. The few remaining hepatic cells were atrophic and disarranged from the effects of the pressure exerted by the encroaching neoplasm. There was considerable extravasation of blood in some areas, which explained the diffuse brownish-colored portions observed in the cross-section of the gross tumor. The round-cell infiltration which was so noticeable in Case I was not observed. In a few areas the tumor cells had undergone distinct hyaline change. Cirrhosis was not observed. Glycogen was demonstrated by Best's carmine stain. A diagnosis of primary carcinoma of the liver was made.

COMMENT

Three types of primary epithelioma of the liver may occur. Only two of these have been described in cattle. In one the neoplastic cells have their progenitors in the biliary epithelioma of the bile ducts with the resultant growth adenomatous in character. Another arises from the parenchymatous cells of the organ. Both of the tumors described here were of the latter variety, and the term "hepatoma" which has been used by certain observers in designating primary carcinoma of the liver could be used at least clinically in designating this type.

The third type of epithelial tumor which may arise from the liver, according to Mallory,¹⁶ is from misplaced cells from the suprarenal cortex. I have not found such a tumor reported in any of the lower animals.

The multiple nodules observed in Case I suggest two possibilities: either that the disease had a multicentric origin, or that the smaller nodules arose secondarily from the primary focus. The clinical, necropsy, and histologic data favor the latter explanation.

The designation of both of these tumors as carcinomas is justified: in Case I by the presence of the aggressive infiltrative manner of growth and many immature cells, many of which revealed mitosis. In Case II the designation of carcinoma is more difficult to defend. Secondary foci were not demonstrated and much of the tumor was

definitely encapsulated by a formidable connective tissue barrier. The mitotic figures, however, and the large numbers of immature cells, with the abundant possibilities for the tumor cells to be carried by the blood to distant situations, make a diagnosis of primary carcinoma of the liver tenable, if not absolutely conclusive.

In man the greater percentage of cases of primary carcinoma of the liver are associated with cirrhosis. Ewing¹⁷ stated that cirrhosis is the chief predisposing factor and that it occurs in about 85 per cent of cases. McIndoe and Counseller¹⁸ also emphasize the probable relationship between cirrhosis and hepatoma and say that it is the main predisposing factor in the disease.

The rôle of cirrhosis in the production of primary hepatic carcinoma is explained on the basis of a regenerative overgrowth of injured or degenerated cells with a resultant loss of restraint and the assuming of autonomous tendencies.

Contrary to the foregoing observations, cirrhosis was not demonstrated in either of the cases forming the basis of this report. Accurate data concerning the incidence of cirrhosis in the various domesticated species are not available, although Hutyra and Marek quote Tschauner as having found three cases of hepatic cirrhosis in 5700 hogs. Although cirrhosis does occur sporadically in the lower animals it does not constitute one of the major pathologic alterations due, perhaps, to the absence in the lower animals of many of the contributory factors frequently associated with the etiology of portal cirrhosis in man. One cannot avoid the inference that primary carcinoma of the liver would be more frequently seen in animals if portal cirrhosis occupied the same relative position in the diseases of animals that it does in diseases of man.

The possible relation of parasitism to primary carcinoma of the liver is suggested from the observations of Roussy and Wolf who reported the presence of nematodes in the liver of cattle affected with primary epithelioma. The liver of sheep affected with similar tumors often harbors parasites, according to these writers.

In these cases it seems reasonable to assume that the toxic products of the parasite and the mechanical irritation of their presence could well incite a degenerative injury of the hepatic cells that would be followed by a regenerative attempt at restoration with resultant cirrhosis and perhaps a neoplastic change in the cells of the parenchyma.

The conclusion one must draw from an examination of the cases reported in the literature is that ordinarily primary carcinoma of the liver is not a tumor of vicious malignancy. Occasionally these tumors cause widespread distribution, but as a rule their activities are limited to the organ responsible for their histogenesis. Occurring as they do in older animals, it is not surprising that serious hepatic involvement should contribute to the general debility so often seen in old cows with subsequent emaciation or perhaps cachexia.

SUMMARY

From a review of the literature one must conclude that primary carcinoma of the liver is not one of the common tumors of the lower animals. Of the domesticated species the dog seems to be the most often affected. The tumors have also been reported in the following species: horse, cattle, sheep, cat, hog, woodchuck and chicken. In one series reported by Trotter one hundred and nineteen cases were found in 39,704 necropsies on cattle.

The true primary carcinoma of the liver arises from the parenchymatous hepatic cell, and while the tumor is occasionally extremely malignant it usually exerts its major influence on the hepatic substance in which it arises, metastasis being the exception rather than the rule.

Two original cases of primary carcinoma of the liver are reported, both occurring in cattle. Metastasis was not observed in either case.

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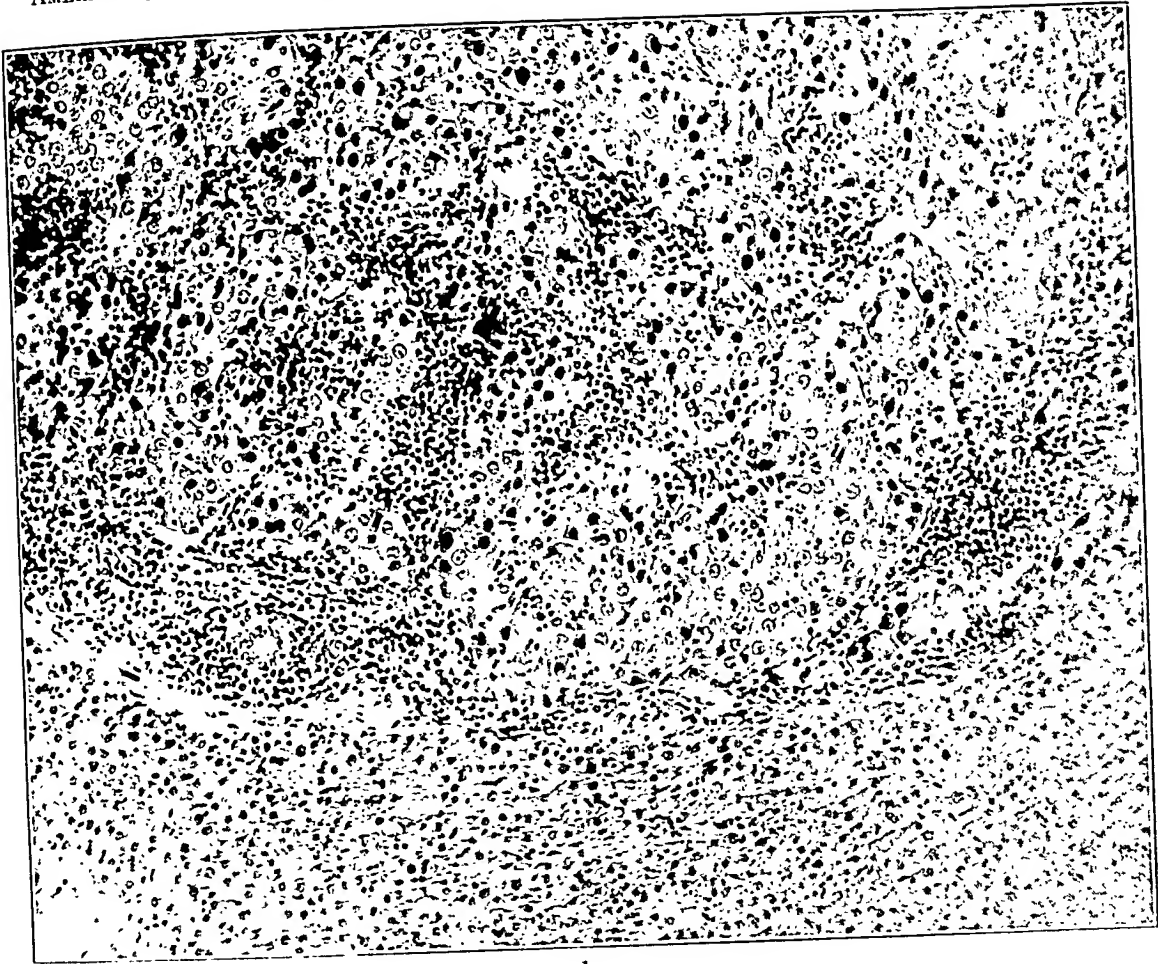
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DESCRIPTION OF PLATES

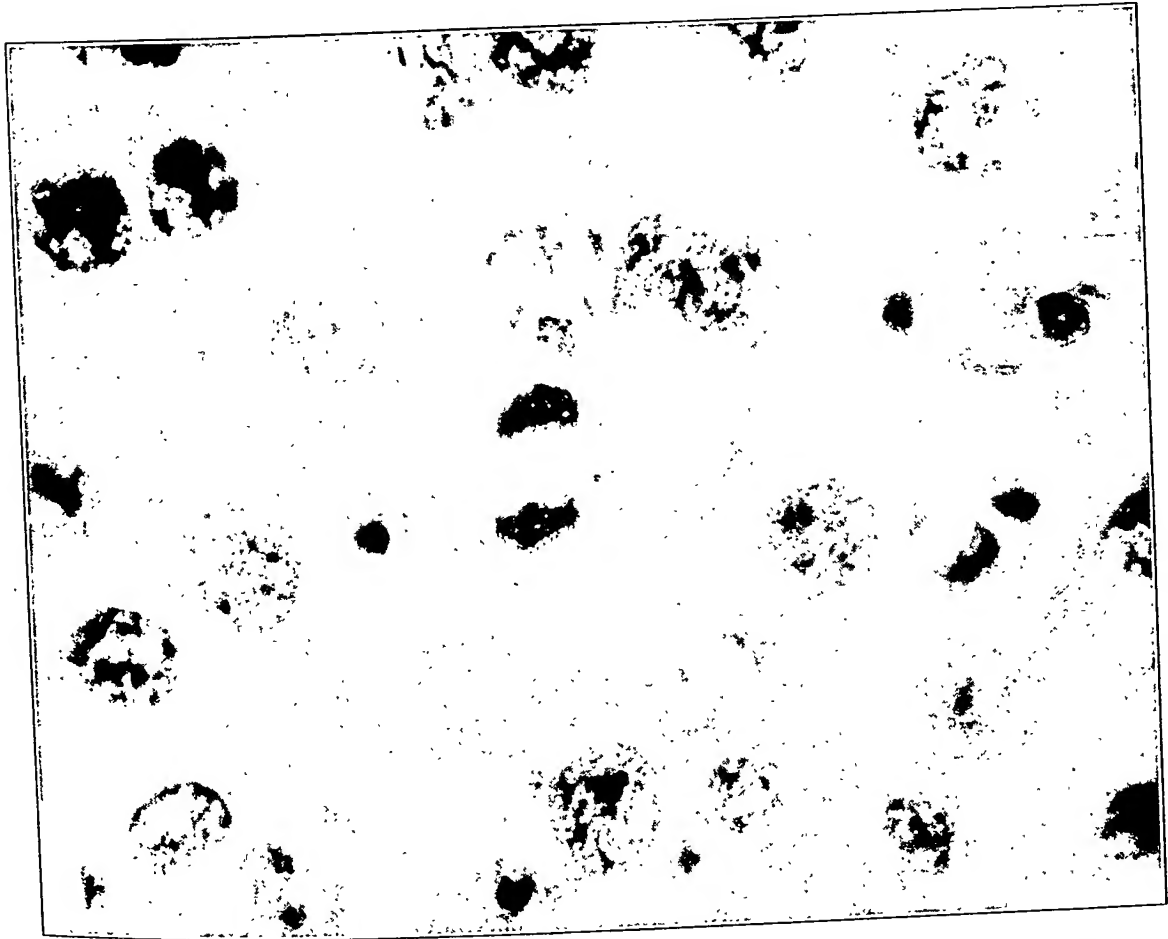
PLATE 137

FIG. 1. CASE I. Primary carcinoma of the liver. Advancing cells of the tumor pushing outward into the tissue which is much compressed and atrophic. The extreme lymphocytic infiltration is apparent. $\times 150$.

FIG. 2. CASE I. Primary carcinoma of the liver. One cell in mitosis. $\times 1350$.



1

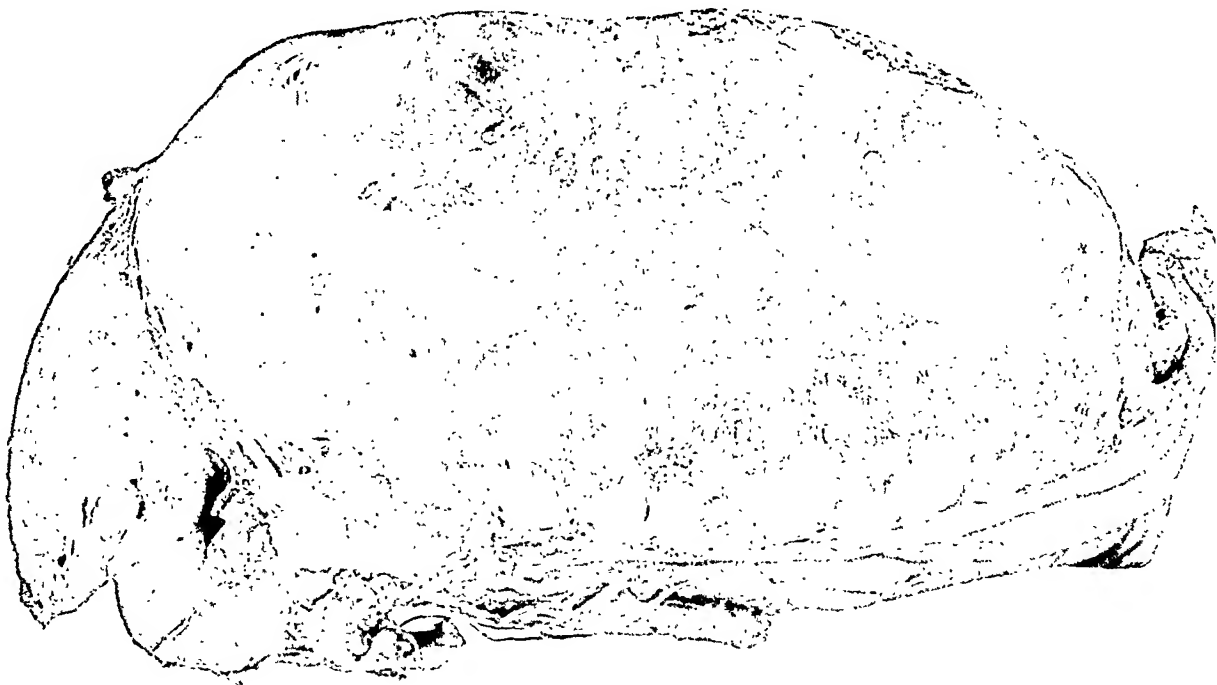


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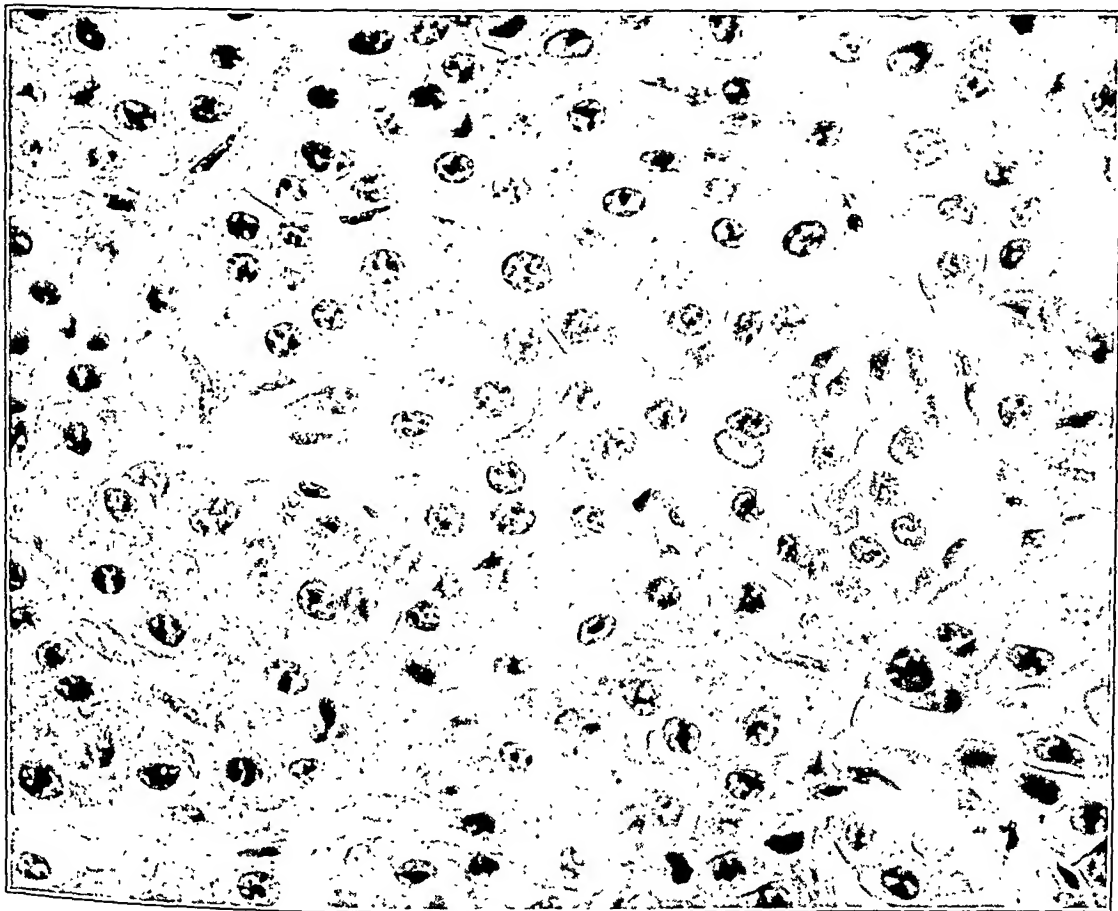
PLATE 138

FIG. 3. CASE II. Primary carcinoma of the liver. A cross-sectional view of the gross specimen. The small amount of liver tissue and the areas of hemorrhage are shown.

FIG. 4. Primary carcinoma of the liver showing the compactness of the tumor cells and the absence of a regular arrangement. $\times 550$.



3



4

an increase of fluidity of this substance, took place, and, as a result of this process, pressure was exerted on the acinus cells, which was injurious to the latter and brought the proliferation to a standstill.

In continuation of the work of the latter authors, we attempted to determine in a quantitative manner the variation in mitotic activity at various periods during the administration of potassium iodide, and furthermore, we wished to determine whether there existed a relation between the quantity of iodide administered and the intensity of the proliferative process produced.

The experiments were carried out on four sets of guinea pigs, weighing on the average between 350 and 450 gm.; some of these were fed with varying quantities of potassium iodide for different periods of time, while others were kept as controls. The first set of animals received a daily dose of 0.01 gm., the second set 0.05 gm., and the third set 0.1 gm. of potassium iodide. The fourth set were kept as controls and did not receive any potassium iodide. The iodide was administered in the form of pills fed by mouth, great care being taken that all of the substance was swallowed. In different groups of animals the feeding was continued for periods of 10, 15, 18, 20 and 30 days respectively, and at the end of each period the animal was killed with chloroform, and both lobes of the thyroid, excluding the isthmus, were immediately removed and at once fixed in Zenker's solution. The glands were cut in complete serial sections and stained with hematoxylin and eosin. The average number of sections obtained for each gland varied between 450 and 500; mitoses were counted in every tenth section in a very exact manner; the number of mitoses thus obtained was multiplied by ten in order to find the total number of mitoses in the thyroid gland. Changes in body weight of the animal during the period of the experiment were also recorded, because, as will be shown in a separate paper, the experiments of Loeb as well as my own show a definite relation between the loss and gain in body weight and the mitotic activity in the thyroid gland. We shall first discuss the changes observed in the number of mitoses in the thyroid epithelium under the influence of potassium iodide feeding; this will be followed by a description of the changes which take place in the thyroid gland under similar conditions.

I. COUNTS OF MITOSES

(A) *Control Animals*: The number of mitoses found in the thyroid gland of our control animals varied in the majority of cases between 135 and 250. (See Table I.)

TABLE I

Mitoses, Control Series

	10 days	15 days	18 days	20 days	30 days
	180	135	440	60	380
	0	190	196	140	120
	200	156	0	40	235
	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
Averages of mitoses	127	160	212	80	245

Of fifteen control animals, in eight the number of mitoses ranged between 135 and 250, in two they were 380 and 440 respectively, and in five they were below 135. The total average of mitoses was 165, with the highest number reaching to 440 and the lowest being zero.

(B) *Counts of Mitoses in Animals Fed with KI for a Period of 10 Days*: Animals that were fed with potassium iodide for ten days showed a relatively slight increase in the number of mitoses as compared with the controls. (See Table II.) There was found furthermore a slight increase in the number of mitoses in passing from animals which were fed with smaller doses of potassium iodide to the animals which were fed with the larger doses. The averages were 280 in the animals receiving 0.01 gm., 370 in those receiving 0.05 gm. and 420 in those receiving 0.1 gm. of potassium iodide.

TABLE II

KI Feeding 10 days

	0.01 gm.	0.05 gm.	0.1 gm.
	200	320	420
	360	420	400
	240	400	440
	320	340	410
	430
	<hr/>	<hr/>	<hr/>
Averages of mitoses	280	370	420

(C) *Counts of Mitoses in Animals Fed with KI for a Period of 15 and 16 Days*: In this group of animals a remarkable increase in mitoses has taken place over the number found in the controls as

well as in the animals fed with potassium iodide for ten days; moreover, the increase is greater, the larger the quantity of iodide administered. (See Table III.)

TABLE III
KI Feeding 15 days

0.01 gm.	0.05 gm.	0.1 gm.
2100	3210	6884
1340	3790	3442
1910	3440
1530	6890
<hr/>	<hr/>	<hr/>
Averages of mitoses 1720	3500	5164

We may therefore conclude that the stimulating effect of potassium iodide on the proliferative activity of the thyroid gland becomes very effective after almost two weeks of iodide feeding and that the effect is greater the larger the quantity of iodide given. The averages found when potassium iodide had been administered for a period of fifteen days were 1720 mitoses, 3500 mitoses and 5164 mitoses in animals fed with 0.01, 0.05 and 0.1 gm. respectively. The highest number of mitoses in this group was 6890 and the lowest number was 1340, the latter figure being found in the animal fed with the smallest quantity of potassium iodide. The number of mitoses in animals fed with 0.1 gm. of potassium iodide was therefore 31 times as great as in the control animals, while it was 21 times greater in animals fed with 0.05 gm. and 10 times greater in animals fed with 0.01 gm. of potassium iodide. The number of mitoses in the animals fed with 0.01, 0.05 and 0.1 gm. of potassium iodide show therefore an approximate ratio of 1:2:3.

Experiments, extending over a period of sixteen days, showed the same results. (See Table IV.)

TABLE IV
KI Feeding 16 days

0.01 gm.	0.1 gm.
1800	3361
1560	5280
<hr/>	<hr/>
Averages of mitoses 1680	4320

Two of four guinea pigs, which were of approximately the same weight, fed with 0.01 gm. potassium iodide showed an average of

1680 mitoses, while two guinea pigs fed with 0.1 gm. showed approximately two and one-half times as much, namely, an average of 4320 mitoses.

(D) *Counts of Mitoses in Animals Fed with KI for 18 Days:* The feeding of potassium iodide to guinea pigs during a period of eighteen days is also characterized by an increased proliferative activity of the thyroid epithelium, which in the case of the smallest dose is somewhat more intense than that observed in the animals fed with potassium iodide during a period of fifteen days. (See Table V.) The relation in this experiment between the number of mitoses found after feeding with 0.01, 0.05 and 0.1 gm. potassium iodide varies according to the ratio of 1:1.2:1.8 respectively.

TABLE V
KI Feeding 18 days

0.01 gm.	0.05 gm.	0.1 gm.
1520	3480	5300
4960	3170	5340
2036	3790	4960
3240	5680
—	—	—
Averages of mitoses 2939	3480	5320

(E) *Counts of Mitoses in Animals Fed with KI for a Period of 20 Days:* In this group we find essentially the same conditions as those described in periods of fifteen and eighteen days. However, in the case of animals fed with 0.05 and 0.1 gm., there was observed a further increase in the number of mitoses. (See Table VI.) In this period the highest counts in mitoses were observed in animals fed with 0.05 and 0.1 gm. of potassium iodide. They exceeded the average counts in any of the previous periods.

TABLE VI
KI Feeding 20 days

0.01 gm.	0.05 gm.	0.1 gm.
2226	4190	8000
2250	4216
2160	4218
—	—	—
Averages of mitoses 2212	4208	8000

But we must consider the fact that only one animal was counted which had received 0.1 gm. of potassium iodide for twenty days and

in which the number of mitoses was 8000. The proportion in counts in the animals fed with different quantities of iodide varied according to the ratio of 1:1.9:3.6. We find then during the third week a most remarkable increase in the number of mitoses throughout the various doses of the iodide feeding, which is greater in cases where larger quantities are fed.

(F) *Counts of Mitoses in Animals Fed with KI for a Period of 30 Days:* In experiments in which iodide feeding was continued for thirty days, the marked proliferation of the thyroid epithelium was lacking. (See Table VII.)

TABLE VII
KI Feeding 30 days

	0.01 gm.	0.05 gm.	0.1 gm.
Mitoses	40	120	600

The average number of mitoses found here was approximately within the range noted in our control animals not fed with potassium iodide. There was perhaps a very moderate increase in the guinea pigs which had received 0.1 gm. of iodide. We may therefore conclude that secondary factors cause cessation of the stimulating effect which is produced with potassium iodide when administered for a period of over two to three weeks.

TABLE VIII
Summary of Results of KI Feeding

Time	Controls	0.01 gm.	0.05 gm.	0.1 gm.
10 days	127*	280	370	420
15 days	160*	1720*	3500*	5164*
16 days	1680*	4320*
18 days	212*	2939*	3480*	5320*
20 days	80*	2212*	4208*	8000*
30 days	245*	40	120	600
*Averages of mitoses	165 (15)	2138 (13)	3729 (8)	5701 (11)

The previous experiments were carried out during the cooler season of the year. An earlier series of experiments, of a similar character to those reported above, were carried out during the summer months. Unfortunately, in the summer series, the glands were not sectioned completely, and consequently the number of mitoses in the entire gland could not be determined accurately. The study

of the sections which were available showed, however, that they agreed with our conclusions as to the stimulating effect of potassium iodide feeding on the thyroid gland. In these experiments also, the greatest numbers of mitoses were found in animals fed with potas-

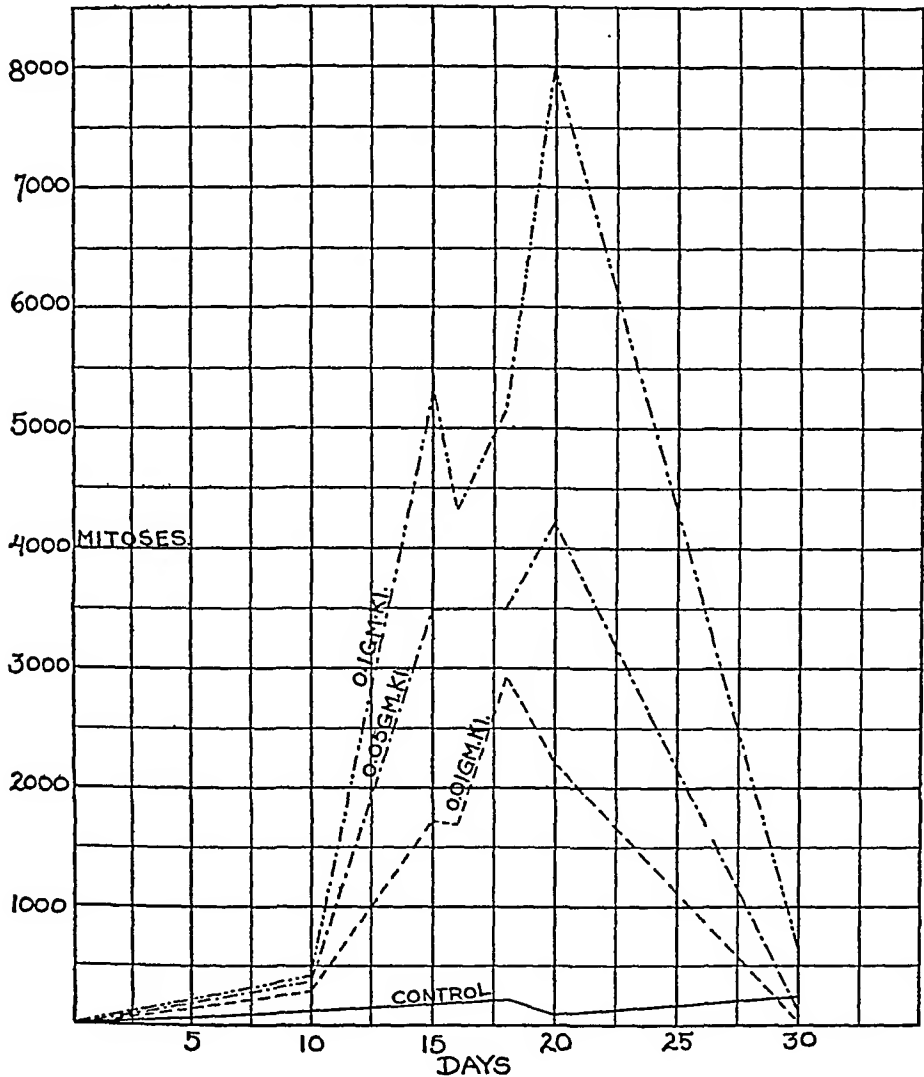


Chart I

sium iodide for about two to three weeks, and furthermore, those guinea pigs which received the larger quantity of iodide showed a greater proliferative activity.

Considering all these experiments we may conclude that potassium iodide stimulates proliferative activity in the thyroid in an extraordinary manner; that the stimulation is very definite two to three

weeks after the feeding of the iodide and that the larger doses cause a greater stimulating effect than the smaller doses within the range of quantities administered by us. Thirty days after the beginning of the experiments secondary factors have begun to become active which counteract the proliferation of the epithelial cells.

Table VIII summarizes the results obtained by us, omitting however the experiments done during the summer, the latter not being of an exact, quantitative nature. The average of mitoses in the control animals is 165, while the number of mitoses in animals fed with the different quantities of potassium iodide for a period of two to three weeks are 2138, 3729 and 5701 respectively. The figures in parentheses indicate the number of experiments in each case.

A graphic representation of the changes taking place in animals under the influence of feeding various quantities of potassium iodide as well as of the conditions found in control animals is given in the chart.

As will be observed, the control curve is very low and relatively uniform. The highest curve is that representing the effect of 0.1 gm. potassium iodide feeding; the maximum of the various curves lies between eighteen and twenty days, the base is reached or almost reached thirty days after the beginning of the iodide feeding.

II. CHANGES IN THE CHARACTER OF THE ACINI, EPITHELIUM AND COLLOID

Control Animals: In the control animals we find small or medium-sized alveoli, lined with cuboidal epithelium and filled with solid colloid; but certain variations occur from this typical picture. In some cases the acini may be larger or smaller than the average, the epithelium may be somewhat flatter or higher, and the colloid softer and filling the alveoli very incompletely. Occasionally also a moderate number of phagocytes may be found in the colloid of various acini. Furthermore, collections of lymphocytes may be noted, in some instances, although these are not frequent occurrences.

Animals Fed with Potassium Iodide: During the first ten days of potassium iodide feeding, no marked changes in the structure of the thyroid gland are observed. In the animals fed for a period of fifteen

to twenty days with potassium iodide, however, some changes may be observed, although they are on the whole slight. The acini, as a rule, are of medium size or perhaps a little larger and regular in outline; the cells are usually somewhat increased in size particularly in cases where many mitoses are found. However, this does not hold good in every instance; at times we find areas where the epithelium is relatively low or flattened out. There is noticeable a slight tendency on the part of the colloid to soften and to retract from the margins of the epithelial lining of the acini. The most striking feature observed, however, in this group of animals, is the very pronounced increase in the number of phagocytes, which we find in the colloid of the acini. This increase in phagocytosis under the influence of potassium iodide has also been noted by Gray and Loeb. At the same time some softening and solution processes take place in the colloid which may go so far that the latter may disappear from the lumen of some of the acini. The phagocytic activity is probably due to changes which the feeding with potassium iodide produces in the thyroid gland.

The histological appearance of the thyroid, obtained from animals that were fed with potassium iodide for thirty days, shows more pronounced changes than those of earlier periods. These changes concern the acini and epithelium as well as the colloid. After thirty days the acini are large and sometimes irregular in outline, owing to the occasional fusion of two or three neighboring acini. Associated with this increase in size and occasional irregularity of the acini is a lowering or even complete flattening of the lining epithelium. The colloid becomes very soft or it may be entirely liquefied. In accordance with the view expressed by Gray and Loeb ^{6,7} we are inclined to consider the changes in the colloid as primary and the changes in the character of acini and epithelium as the result of mechanical pressure exerted by the increased contents of the acinus, on the wall of the latter. The phagocytic activity which was increased in the previous period, is less marked in the acini thirty days after the beginning of the KI administration.

Maintenance of Body Weight: In the experiments of Loeb on the hypertrophy of the thyroid gland, there was noticed a relation between the degree of hypertrophy and the changes in the weight of the animal which took place during the course of the experiment. A gain in weight during this period was a condition favorable to

hypertrophy, while a loss in weight had the opposite effect.⁸ The same relation was observed by us in our present experiments; the thyroid epithelium showed more active proliferation in the former than in the latter case. We shall discuss this relationship in a subsequent paper.

DISCUSSION AND CONCLUSIONS

We consider the results which we obtained in our experiments as very significant. They show quite conclusively that potassium iodide, when given to normal guinea pigs in the manner we described above, induces a pronounced proliferation of the acinus cells in the thyroid gland, as evidenced by the marked increase in the number of mitoses. The increase in cell division, resulting from the iodide feeding, is most intense between the fifteenth and twentieth day of the administration of this substance. It is far less pronounced or is absent during the first ten days of the iodide feeding, and it decreases again very markedly when treatment is prolonged to thirty days. At this latter period the proliferation is even less marked than at the tenth day. It is furthermore noted that, within the range of our experiments, the greater the dose of the iodide administered to the animals, the more intense is the degree of mitotic cell division. This quantitative relationship between the dose of potassium iodide given and the intensity of cell proliferation is brought out very clearly in our tables.

Structural changes in the thyroid as far as they relate to the character of the colloid, of the acini and the lining epithelium, are lacking in the first ten days of the iodide feeding; between the fifteenth and twentieth day, the changes observed are very slight and consist in a somewhat softer colloid and perhaps a slightly higher epithelium, while the acini remain of about the same size. During this stage we notice, however, the appearance of a very large number of phagocytes in the colloid. After thirty days of iodide feeding, there occur more appreciable changes; the acini become large, irregular and often fuse together, the colloid is soft and edematous and the cells are low and flattened out. The phagocytes now tend to diminish or disappear entirely.

SUMMARY

1. A quantitative estimation of the proliferative activity of the thyroid gland has been carried out, by counting the number of mitoses present in the normal gland as well as in the glands of guinea pigs fed with various quantities of potassium iodide during different periods of time.

2. The number of mitoses found in thyroids obtained from animals fed with potassium iodide within the first three weeks after the beginning of the feeding is by far greater than that observed in the controls. The increase in the number of mitoses is only slight during the first ten days. It becomes very pronounced between the fifteenth and twentieth day of the iodide feeding, and lessens again when feeding is continued for thirty days.

3. Within the range of quantities of the substance used by us, larger doses of potassium iodide call forth greater proliferative activities of the thyroid epithelium than smaller doses.

4. At the same time the colloid becomes slightly softer and the phagocytic activity within the colloid increases after potassium iodide has been fed for fifteen to twenty days; the epithelium, however, changes very little in height. At the end of the thirtieth day, the acini enlarge and become irregular, the colloid becomes watery and the epithelium flattens out; the number of phagocytes also diminish. We attribute the flattening of the acinus epithelium, which is observed in the fifth week, to the pressure exerted on the epithelium by the increase in volume in the colloid resulting from the absorption of water at this period.

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SCIENTIFIC PROCEEDINGS OF THE
TWENTY-EIGHTH ANNUAL MEETING
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AMERICAN ASSOCIATION OF PATHOLOGISTS
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MAY 1, 2 AND 3, 1928.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

SEROLOGICAL DISCORD IN LATENT AND TREATED SYPHILIS. Adele E. Sheplar and Morris A. Lyons (by invitation) and Ward J. MacNeal, New York City.

Abstract. Examination of identical specimens of serum by the methods of Wassermann, of Vernes and of Kahn discloses frequent discordant results, especially in serum from syphilitics under treatment or in a latent stage of the disease. Such observations as the few selected for the following table are not unusual.

<i>Wassermann</i>	<i>Vernes Reading</i>	<i>Kahn</i>	<i>Remarks</i>
—	71	4	Tertiary syphilis
—	22	—	Angina pectoris
—	0	4	Colloid goiter
—	0	4	Tertiary syphilis
4	30	—	Syphilis
3	0	4	Tertiary syphilis
4	52	—	Tertiary syphilis
2	0	4	Tertiary syphilis
4	22	—	Syphilis

Furthermore, in testing the blood of the same patient at intervals it is found that the reading by one test may ascend while that of another may be lowered.

In all three methods the reading is an attempt to express the quantity of lipoidophilic reagin present in the patient's blood, which will enter into reaction with alcoholic extract of heart muscle. The discordant results here recorded indicate that the syphilitic reagin is not a single substance but probably a mixture of various substances, whose quantitative relations may determine a high reading when tested by one method and a low reading by another.

It is suggested that variations in composition of the reagin mixture may depend not only upon the stage of the disease but also upon the character of the tissues in which lesions are situated.

Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) I was very much interested in Dr. MacNeal's report because it confirms in many respects the experience we have had in comparing the work done in different laboratories. Although we have not worked with the Vernes or Kolmer tests, we have recently had some experience with the Kahn precipitation reaction, which indicates that the test is not quite so reliable as the complement-fixation test with the cholesterinized antigen, unless the latter is too delicately adjusted. If one compares the reactions obtained with the Wassermann tests and Kahn tests made in different laboratories, it will often be found that quite discrepant results have been secured due to technical errors and variations in the adjustment or control of the tests. We have found it particularly essential in our comparisons to be sure that the test with the cholesterinized antigen is not too sensitive and yet is sufficiently delicate. The only way I could rely upon the test being properly adjusted was

by studying the results of comparative tests made in a number of laboratories, which, from time to time, have been asked to coöperate. Such a comparison gives one a valuable basis for the evaluation of the accuracy of serological examinations. If the results are plotted, here and there one laboratory will miss a reaction, but those obtained by the majority furnish a very fair basis for the control of the accuracy of your own test.

(Dr. A. S. Giordano, South Bend, Ind.) I have tried the Kahn, Kline, and Kolmer tests and even though this is rather preliminary, comparing it with the Kolmer, the Kline is far superior to the Kahn and runs more parallel to the Kolmer than the Kahn. There is 10 or 15 per cent difference in the two.

(Dr. MacNeal, closing.) We have taken special precautions to avoid confusion of specimens and technical errors, which are obviously suggested by such discordant results. I am myself convinced that there is something else besides technical error involved here. I would like to decry the too great faith in any one serological test or any group of serological tests. On the whole, it seems to me the syphilologists are putting too much confidence in serological examinations. Some men will not use them at all but they are unwise in this. Serological tests are a great help when used in the proper way. The positive Kahn, positive Wassermann or positive Vernes reading may be regarded as absolute proof of syphilis by enthusiasts so that they tend to neglect other evidence. One should not be so slavishly enthusiastic about any one method. A proper clinical record including the results of serological tests from time to time is still the best guide.

THE DIFFERENTIATION BETWEEN INFECTION AND IMMUNITY BY SERUM AGGLUTININ ANALYSIS. I. Forest Huddleson (by invitation), East Lansing, Mich.

Abstract. A method is described for differentiating serum agglutinins occurring in the host during the presence of a pathogen, from those which may be demonstrated in the serum after the passing of the inciting agent, or arising as a result of preventive inoculation.

The differentiation of serum agglutinins by the described method depends upon the following factors: the employment of a specific antigen suspended in 12 per cent sodium chloride solution and the addition of the antigen to 0.1 cc. of serum on a glass plate without mixing. The differentiation is determined after a lapse of one minute by the type of flocculation.

The method has been applied to the study of Bang's abortion disease in the cow and undulant fever in man.

Discussion

(Dr. A. S. Giordano, South Bend, Ind.) I would like to ask Dr. Huddleson if he would make some statement about the preparation of antigen. This is a very important question when applied to humans, particularly when doing routine agglutination tests on blood. We are often confronted with the question as to the determination of agglutination, whether or not the patient has had the disease, or is at present affected by the disease. We would like to ask him whether or not the antigen is the same as he uses in making agglutination tests as described in his previous article.

(Dr. Huddleson, closing.) The antigen for the differential test is prepared very much like the antigen for the rapid macroscopic agglutination test described in a recent number of the *Journal of Infectious Diseases*, the chief difference being the elimination of the boiling procedure.

STUDIES ON THE ANTIGENIC PROPERTIES OF ULTRAMICROSCOPIC VIRUSES.
E. W. Schultz, Stanford University, California.

Abstract. Though specific neutralizing antibodies were easily demonstrable in antisera experimentally produced in rabbits against the viruses of vaccinia, rabies and herpes, no evidence whatever of specific complement fixing or precipitating antibodies could be elicited in these sera. These facts together with the general trend of the results already reported in the literature on these and other ultraviruses, raise the question of whether perchance the ultraviruses, because of their relatively low physical and chemical organization, as suggested by recent ultrafiltration studies, may not, like the true toxins, exhibit antigenic properties of a more restricted and specialized character than those which we know to hold, for example, for bacteria and other complex proteinaceous bodies.

Discussion

(Dr. John Reichel, Philadelphia.) I would like to ask Dr. Schultz how he determined the rabicidal properties of the immune serum.

(Dr. Schultz.) A suitable suspension of rabies virus was brought together with the inactivated immune serum in a test tube and incubated at 37.5° C for four hours. This mixture was then injected intracerebrally into a rabbit. At the same time a control rabbit received a similar injection of the virus treated in an identical manner with inactivated normal rabbit serum.

(Dr. Reichel.) I am interested in the fact that neutralization of the virus was obtained with inactivated immune serum. In some studies which I have made I have been able to demonstrate rabicidal action only when I used fresh serum. Inactivated serum had no effect on the virus.

(Dr. Schultz, closing.) We obtained definite rabicidal action with *inactivated* immune sera. That rabicidal action may be obtained with *inactivated* immune sera, is, I believe, also quite well supported by the literature.

ON THE MECHANISM OF OPSONIN AND BACTERIOTROPIN ACTION. I. EXPERIMENTS WITH ACID-FAST BACTERIA. Stuart Mudd, Baldwin H. Lucké, and (by invitation) Morton McCutcheon and Max Strumia, Philadelphia, Pa.

Abstract. These experiments represent the first stage of a study directed toward analysis of the mechanism of opsonin and bacteriotropin action in physical-chemical terms. They are intended to answer the question: "What changes do sera effect in acid-fast bacteria in preparing them for phagocytosis?"

Rabbit-immune sera have been prepared against a variety of acid-fast bacteria. The bacteria, sensitized by serial dilutions of homologous and heterologous antisera, or by normal sera, have been studied in the following reactions. (1) The bacteria remain in serum dilutions over night and the *agglutination* is then read. (2) The serum-bacterial mixtures are strongly centrifugated and the sediment resuspended by shaking; this *resuspension* reaction gives an estimate of the cohesiveness of the bacteria. (3) The sensitized bacteria are washed and their wetting or interfacial tension properties are then estimated in the *interface* reaction. (4) The electrical charge of the washed, sensitized bacteria is determined by *cataphoresis*. These four reactions together give a picture of the surface properties of the bacteria. (5) Meanwhile mixtures of rabbit leucocytes with (a) washed, sensitized bacteria, or with (b) bacteria and serum dilutions, are rotated in stoppered vials on a Robertson agitator. Smears are made from each mixture, stained, and a hundred leucocytes in each smear are observed

microscopically. The percentage of leucocytes which have taken up bacteria is recorded.

Immune sera reacting with acid-fast bacteria cause agglutination, phagocytosis, increased cohesion of the bacteria, reduced bacterial surface charge, and change from a bacterial surface readily wet by oil to a surface readily wet by water. The most important relationship thus far brought out is that in practically every instance in which an immune or normal serum has caused increased phagocytosis it has caused in approximately corresponding degree the above-indicated changes in surface properties. Similarly when immune sera have reacted with the bacteria so as to produce surface changes, corresponding increase in phagocytosis has usually resulted. Thus, of twenty-one series in which bacteria were treated with homologous sera, nineteen showed surface changes and bacteriotropin action in roughly corresponding degree; of sixteen heterologous series there was satisfactory correspondence between surface changes and bacteriotropin action in twelve.

Failure of correspondence was due to failure of bacteriotropic action with old sera (approximately 18 months ageing). Another anomaly was observed with these aged sera. Striking phagocytic prezones were regularly obtained when bacteria were treated with aged sera homologous with them; phagocytosis reached a maximum in some instances, not as with fresh sera in the highest serum concentrations, but in serum concentrations as low as one tenth of one per cent.

The serum of four rabbits has been followed by similar tests at weekly intervals during the course of active immunization. Striking correlation was found between the bacterial surface changes and the bacteriotropin effects produced by the sera of these animals.

The correlation between bacterial surface changes and opsonin effects produced by fresh normal rabbit sera was satisfactory with eleven out of twelve sera. When the sera were heated for 30 minutes at 56° C their effects on the bacterial surfaces were reduced but by no means abolished; on the other hand their opsonic effects were lost in nine out of sixteen cases. Normal human sera altered the bacterial surface properties but did not induce phagocytosis by rabbit leucocytes.

In summary, then, sera which in these experiments have increased the phagocytosis of bacteria have concomitantly increased the cohesion, decreased the surface charge and altered the wetting properties of the bacteria. Similarly, but with certain exceptions, when sera have altered the surface properties of bacteria as indicated they have increased phagocytosis. The exceptions have practically all been with aged or heated sera.

Discussion

(Dr. R. R. Mellon, Pittsburgh.) I would like to ask Dr. Mudd whether these organisms are sensitized and consequently reduced in charge or whether the flocculation found occurred spontaneously.

(Dr. Mudd.) The cohesive factor seems to be more important in affecting agglutination and seems to be more variable with acid-fast bacteria than with other species. Flocculation in our experiments has not been spontaneous but has been with sensitized bacteria whose surface potential difference has been reduced and whose cohesiveness has been increased by sensitization. Because of the difficult and irregular agglutinability of these strains we do not regard the ordinary agglutination reaction as of much importance with them. By centrifug-

gating the sensitized bacteria and then resuspending them, however, a fair estimate of their relative cohesiveness in the various serum dilutions can be obtained.

(Dr. Mellon.) Would you say, then, that they are perfectly sensitized?

(Dr. Mudd, closing.) I think that probably the data indicate that the film of antibody proteins is possibly incomplete, that it is not a complete film.

AQUEOUS-LIPOIDAL PHASE REVERSAL IN THE CELL WALL OF 'S' AND 'R' BACTERIAL FORMS: ITS BEARING ON THEORIES OF ELECTRICAL P. D. IN SUSPENSION STABILITY. Ralph R. Mellon, Pittsburgh, Pa.

Abstract. The large number of studies on the electric charge of bacteria especially in its relation to spontaneous agglutination have never been able to evaluate the rôle of the surface tension factor. This, because of no satisfactory method for measuring bacterial interfacial tension, the method advocated by Northrup and DeKruif being quite inaccurate. The result has been an undue appreciation of the charge factor at the expense of the surface tension factor.

Several years ago one of our papers called attention to this aspect of the situation. The ground for our position rested on experiments with sodium oleate whereby certain strains of high interfacial tension could be readily emulsified with this reagent. In this connection we also showed that the principle of ion antagonism was applicable, which indicated the presence of an aqueous-lipoid system in the cell wall that was reversible by the cations Na and Ca where the anion Cl remained the same.

This contention is now susceptible of experimental demonstration with certain strains, *in which instances* the electric charge appears to play a minor or even an insignificant rôle in suspension stability. In a word, the experiments are as follows: The Ca ion brings the lipid phase to the external surface of the bacterial cell wall, as can be shown by the fact that the organism will pass from an aqueous solvent to lipoidal solvents. The effect of the Ca appears to be a maximal one, and the effect of the solvent a quantitative one, depending on the relative solubility of the lipid in the aqueous solvent. On the other hand, in the presence of the Na ion alone, *under no known circumstances* will the organism leave an aqueous menstruum for a lipoidal one.

These results assume a heightened interest in the light of the recent work of March, who concludes essentially that the greatest electrical P. D. ever recorded with colloidal particles is less than one-twentieth of that necessary to bring about significant mutual repulsion of particles similarly charged.

Discussion

(Dr. S. Mudd, Philadelphia.) Northrup and DeKruif made an important advance in analysis of the mechanism of agglutination when they showed that it depended upon two factors: a surface electrical potential difference tending to prevent collisions between the bacteria and a cohesive force tending to hold them together once they have collided. It would seem a step backward to try to eliminate consideration of the surface potential difference and explain the flocculation only in terms of cohesion as surface tension. Moreover, a surface largely lipoidal does not necessitate spontaneous agglutinability. Many of the acid-fast strains have a surface rich in lipid and yet form stable suspensions in 0.85 per cent NaCl solution.

(Dr. Mellon, closing.) I am sorry that I stressed this thing enough to give Dr. Mudd or anyone else the impression that I was holding any special brief

for surface tension effects to the entire exclusion of other surface factors, such as potential differences. The experiments here presented are intended first to emphasize a fact apt to be forgotten, *viz.*, that there is no method for measuring the interfacial surface tension of a bacterium — therefore when we draw conclusions from potential difference observations we must always assume that the surface tension factor is behaving in accord with the stipulations of Northrup and DeKruif. But it so happens that in this strain we have an organism where the interfacial tension is so high as to be revealed by the solubility methods employed. If we were not aware of this fact, we should conclude that the drop in P. D. with CaCl_2 below the critical level explained the agglutination. Now since the action of the CaCl_2 was essentially to bring the lipid to the surface of the organisms, thus virtually converting them into oil droplets, why should we invoke potential difference as an explanation, at least any more than we should invoke it to explain why oil and water do not mix? This is not inconsistent with the view that the P. D. action is a dispersive one, but under conditions such as the above, it would need be many times greater than the measurements to be taken seriously.

OBSERVATIONS ON THE INTESTINAL BACTERIOPHAGE IN THE SPECIFIC INFECTIOUS DISEASES. D. M. Cowie and (by invitation) Henry G. Poncher, Ann Arbor, Mich.

(Abstract not received.)

IMMUNOLOGICAL STUDIES IN RELATION TO THE SUPRARENAL GLAND. David Perla and (by invitation) J. Marmorston-Gottesman, New York City.

Abstract. Hemolysin formation in normal and suprarenalectomized rats was studied. A single intraperitoneal injection of 1 cc. of a 10 per cent suspension of sheep cells in normal adult albino rats results in an optimum high hemolysin titer five days after injection, which gradually falls, disappearing at about the third week. Ten times this amount produces lower hemolysin titers.

Bilateral suprarenalectomy in rats subsequently injected intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells at various intervals after operation (48 hours, 7, 14 and 28 days) resulted in a depression of hemolysin titer during five weeks following the operation, the depression being most marked during the first week. Bilaterally suprarenalectomized rats injected intraperitoneally two weeks after operation with 1 cc. of undiluted sheep cells have hemolysin titers higher than do normal rats. The quantity of antigen necessary to yield the maximum titer in suprarenalectomized rats two weeks after operation is ten times the quantity necessary to yield the same titer in normal rats. Traumatization of the perisuprarenal tissue in rats produced the same effect on the antibody-forming capacity as suprarenalectomy.

Further, the effect of adrenalin on hemolysin formation in normal rats was studied. Injections of adrenalin in amounts of 0.4 mg. per kilo rat per day in divided doses in normal rats for three days prior and four days subsequent to injection of 1 cc. of a 10 per cent suspension of sheep cells, produced a marked depression in hemolysin formation. The same daily amounts of adrenalin injected during four days subsequent to the sheep cell injection produced a depression to a lesser degree. Adrenalin injected in the same daily amounts but only during one day prior and two days subsequent to the injection of red blood cells had no depressing effects.

AN EXPERIMENTAL STUDY OF THE ACTION OF *B. Welchii* TOXIN ON BONE MARROW. John C. Torrey and (by invitation) Morton C. Kahn, New York City.

Abstract. This study is in continuation of our investigation of the anemia produced in laboratory animals by a potent *B. welchii* toxin. In previous communications attention has been directed to the abnormally high *B. welchii* counts in fecal specimens from pernicious anemia cases. It has also been shown that a transitory anemia with a blood picture strongly suggestive in a number of features of the pernicious type may be produced in monkeys by intravenous inoculations of such a toxin, and further there was reported evidence of the absorption of this toxin from the gastro-intestinal tract of monkeys which had been treated with a weak solution of sodium fluoride to induce a catarrhal inflammation. The present study is concerned with the action of the toxin on the bone marrow following direct inoculation.

The *B. welchii* toxin was prepared by growing the organism in casein-digest broth in sealed tubes as described heretofore. We have found that 2 cc. of fresh rabbit blood per 30 cc. of medium may be substituted for the pigeon muscle. The twenty-four-hour culture was rendered sterile by passage through a Berkefeld filter. Under ether anesthesia 0.5 cc. of the filtrate (1 M.L.D. for a pigeon) was inoculated into the tibial marrow of each of a series of rabbits and of a monkey and the blood and marrow changes were followed for varying lengths of time. In contrast to the immunity sooner or later following one or more intravenous inoculations of the toxin, a single intramarrow injection has a progressive injurious effect causing a severe chronic anemia with very marked loss in weight and frequently a fatal issue. Also the inoculation into the marrow of one bone caused the same type of degenerative changes in almost or quite equal degree in marrows of bones far removed therefrom. In view of the fact that the intravenous inoculation of the same dosage or a series of increasing amounts of toxin would not cause marrow changes of this character or degree, it is difficult to explain the apparent whole involvement of the marrow system following toxin injury to a single bone marrow.

No animal inoculated in this way has made a recovery. Two rabbits died after 74 and 75 days respectively. Others were observed for from 100 to 112 days and were very weak and anemic after that period. Within 24 hours following the inoculation the hemoglobin and the erythrocyte count were reduced about 50 per cent. From this time on the erythrocyte count ranged from 1.5 to about 2.5 million and the Hb from 50 to 60 per cent, except at one or more periods of marked remission. About 10 days following the inoculation the color index rose above 1.0 and stayed there until about two weeks before the death of the animal. Morphological and tinctorial changes in the blood were, on the whole, not quite so pronounced as those following intravenous toxin inoculations. The attempt at blood regeneration was very strikingly shown in the case of three rabbits inoculated into the tibial marrow with 0.5 cc. of the toxin at the same time. The remission occurred in all three simultaneously and seven weeks following the inoculation. This lasted not over a few days during which the erythrocyte counts rose to nearly four million and the color indices dropped below 1.0.

As stated above, inoculation of the *B. welchii* toxin into one bone marrow causes a progressive deterioration of apparently the whole marrow system. This process starts within 18 hours with an atrophy of the marrow cells, ingress of many polymorphonuclear leucocytes and degeneration of the giant cells. After 12 days there was an extremely marked atrophy of the marrow cells, a marked increase and subsequent degeneration of the fat cells, but the most striking

feature was the great abundance of polymorphonuclear leucocytes. At the terminal stage the marrows were a much darker red color than in the normal rabbit. Microscopic examination showed there had occurred in the marrow of the long bones on both sides of the body a replacement of fat and lymphoid cells by a peculiar homogeneous granular material. The few remaining cells were mostly rather large, hyperchromatic granular mononuclears of the myelocyte type. No definite traces of forming red blood cells could be found.

Two control rabbits inoculated with 0.5 cc. of the sterile medium into a tibial marrow were almost unaffected by the treatment. After two months they had gained in weight. The blood examinations showed little or no deviation from the normal. The marrows at necropsy had the same color and appearance as in normal rabbits and microscopically exhibited no change. The medium itself was thus eliminated as a causative factor in the marrow and blood deterioration.

Discussion

(Dr. W. M. Simpson, Dayton, O.) In view of the fact that the reaction of the bone marrow to *B. welchii* toxin is not a local one, it would seem that the entire reticulo-endothelial apparatus might be similarly involved. I would like to know what changes occurred in the spleen and liver; whether there was reticulo-endothelial hyperplasia and hemosiderin deposition?

(Dr. Kahn.) I have nothing further to add to Dr. Simpson's remark. I made no further observation on the spleen or other organs and I cannot give any further definite information.

(Dr. Ottenberg, New York City.) If it is injury to bone marrow, how can one explain the very sudden drop in 24-48 hours of the hemoglobin of the red cells.

(Dr. E. E. Ecker, Cleveland.) I would like to ask whether or not these reactions are specific or non-specific?

(Dr. Kahn, closing.) There is undoubtedly some hemolytic activity in the blood system after intravenous injection. You will get a marked drop in erythrocyte count after injection into the blood stream or the marrow. As far as the production of macrocytes is concerned, marked variations in the size of the erythrocytes were induced by injecting *B. welchii* toxin into the marrow and round to oval macrocytes were frequently observed. As far as other anaerobes are concerned, changes induced by injection of *B. tetanus* toxin were not as marked as those by the *B. welchii* according to the reports of other observers. Also results with staphylococcus were not as apparent as those with *B. welchii*. No observations on reticulated erythrocytes were made.

EXPERIMENTAL INOCULATION OF CHICKENS WITH LYMPH NODES OF HODGKIN'S DISEASE. Elise S. L'Esperance, New York City.

(Abstract not received.)

STUDY ON LONGEVITY OF DIPHTHERIA CULTURES. Henrietta Calhoun, Rockford, Ill., and Henry Albert, Des Moines, Iowa.

(Abstract not received.)

IS LYSIS OF BACTERIA AN ESSENTIAL PART OF THE PHENOMENON OF BACTERIOPHAGY? J. Bronfenbrenner and (by invitation) D. Hetler, New York City.

Abstract. At the meeting of this Society last April, we presented experimental evidence suggesting that lysis of bacteria in the presence of phage may be the direct result of the rupture of bacterial cells, due to an increased uptake of

water from the medium. If this conception is correct, one should expect that in all cases where the swelling of bacteria is prevented, there should be no lysis, and conversely, whenever, under the conditions of the experiment, lysis in the presence of phage is prevented, there should be no swelling of bacteria. These predictions were tested experimentally, taking advantage of the fact that under certain conditions lysis in the presence of phage can be prevented at will.

Susceptible bacteria were seeded on the surface of solid media of different composition, and subsequently droplets of active phage were deposited at various places on the seeded surface. At suitable intervals, contact impressions were taken from the spots on which bacteriophage was deposited. It was found that whenever lysis took place, bacteria invariably underwent swelling. Conversely, all contact impressions taken from spots where phage was deposited, but where lysis did not take place, showed that the bacteria failed to swell.

Thus far, we have attempted to prevent lysis by means of (1) addition to the medium of antibacterial sera; (2) subjecting the cultures to the effect of high temperatures ($42-45^{\circ}\text{C}$), and (3) increasing the concentration of agar or gelatin in the medium.

The mechanism responsible for inhibition of swelling and visible lysis in the first two instances remains for the present unknown. From the fact, however, that bacterial growth under the droplets of phage failed to show any evidence of being affected by its presence, we are inclined to consider that bacteria themselves have undergone some change under the conditions of the experiment, whereby all action of phage on them was excluded. The failure of bacteria to swell and undergo visible lysis on media containing high concentrations of agar or gelatin, we think is directly due to the competition for water between the medium and the bacteria. In this experiment phage undoubtedly exerted its effect on bacteria (even in the absence of lysis) as evidenced by the fact that bacterial growth over the spots where phage was placed greatly exceeded that present on the surrounding medium. Furthermore, when the portions of medium on which phage was deposited were cut out and the amount of phage present in them was determined, it was found that it had undergone some increase in concentration, as compared with the control. (Phage deposited on sterile medium.) It appears that in addition to showing the intimate relation existing between the swelling and the subsequent lysis of bacteria, these experiments confirm earlier observations concerning the regeneration of phage in the absence of lysis. However, the regeneration of phage in these experiments was only partial, and in no instance approached the rate of regeneration observed by us in similar experiments on the media containing lower concentrations of phage or gelatin, and therefore the possibility that some individual bacteria may have undergone lysis cannot be excluded.

The conclusions we draw from these experiments are: that lysis is not an essential part of the phenomenon of bacteriophagy; that under certain experimental conditions the presence of phage is plainly demonstrable in the absence of lysis; that lysis proper is the direct result of the uptake of water by the bacteria, and that whenever this uptake of water is interfered with, either through the changes induced in the bacteria themselves, or in the medium, the swelling and lysis of bacteria are prevented.

Discussion

(Dr. J. W. Jobling, New York City.) I should like to ask Dr. Bronfenbrenner if he varied the salt content of his bacteriophage; if that in itself would have the

same effect as growing the organisms on the media, in other words, change the osmotic effect in that manner?

(Dr. Bronfenbrenner, closing.) It has been observed that when bacteria are grown on media containing high concentration of salts, they contain correspondingly high concentration of salts in their protoplasm. Such bacteria undergo rapid swelling and lysis when transferred to highly dilute media. Inversely, it may be expected that if swollen bacteria, as those in our experiments, are transferred to media containing high concentration of salts they will lose water and will not burst. We tried to carry out such experiments using various salt and sugar solutions, but did not obtain conclusive results. Such experiments are very difficult to perform due to the fact that swelling of bacteria and particularly the bursting of swollen bacteria occurs very suddenly and rapidly.

THE RESORCIN FLOCCULATION TEST FOR ACTIVITY OF TUBERCULOSIS. Adelaide B. Baylis (by invitation) and Ward J. MacNeal, New York City.

Abstract. The blood serum during active tuberculosis is altered in such a way that various reagents will produce a cloud in it. Daranyi employed ethyl alcohol for this purpose. More recently various phenols have been shown to react with tuberculous human serum to produce a flocculation. Vernes employs a 1.25 per cent solution of chemically pure resorcin in doubly distilled water, mixing this with an equal volume of the patient's serum and reading the optical opacity in his photometer at once and again after four hours at 25° C. The difference between the two readings is less than 15 in normal persons, from 15 to 30 in relatively quiescent tuberculosis and above 30 in actively progressive tuberculosis. The test is not specific in a bacteriological sense. In fact, high readings are found for a short time in the primary stage of syphilis and frequently in advanced neoplastic disease. We have also observed high readings in active pneumonia and in pulmonary abscess.

More recently, Baylis has devised a simple technic which does not require the photometer. The resorcin solution and the serum are mixed by pouring from one test tube to another and the mixture is left in the room four hours and then overnight in the refrigerator. The visible precipitate is read roughly and recorded as minus, plus minus, plus, two plus, three plus, or four plus. It is also possible to distinguish an atypical flocculation in which the flakes are coarse and voluminous. This atypical reaction appears to be associated with other conditions than tuberculosis, especially with pulmonary abscess and advanced malignant neoplasm.

These tests promise to be of considerable value in measuring the activity of a known tuberculous process and, when due regard is given to their limitations, they are of some value in differential diagnosis.

THE FATE OF TUBERCLE BACILLI IN VARIOUS ORGANS OF THE RABBIT. Max B. Lurie (by invitation), Philadelphia, Pa.

Abstract. In this study an attempt was made to follow the fate of tubercle bacilli in the lung, liver, spleen, kidney and bone marrow of rabbits infected with large and small doses of human and bovine tubercle bacilli intravenously by determining the number of colonies of tubercle bacilli that can be recovered from comparable quantities of tissue on egg media at varying intervals during the course of infection.

It was found that the original distribution of the bacilli to the several organs

follows the distribution of particulate matter, namely, in the following order: spleen, liver, lung, bone marrow and kidney per gram of tissue.

At first the tubercle bacilli, both of the human and bovine types, grow in all the organs without any effective opposition. The rate of growth of both types of bacilli differs greatly in the various organs. The human type of tubercle bacillus grows faster in the several organs than does the bovine type. This initial growth is followed by a more or less complete destruction of the bacilli in all the organs of the rabbits infected with the human bacillus, while in the bovine infected animals the destruction is more or less complete in the liver, spleen and bone marrow and multiplication continues without effective opposition in the lung and kidney until the death of the animal. The time at which destruction appears in the several organs depends on the organ in question, the type of bacillus used, and the infecting dose. The liver, spleen and bone marrow show the earliest destruction. The lung and kidney destroy the bacilli later if at all. Destruction is apparent earlier in the human infection and later in the bovine infection. In rabbits infected with the human type of bacillus the destruction in the liver, spleen and bone marrow is brought about earlier with a large dose and later with a small dose. The degree of destruction in a given time is more complete in the several organs in rabbits infected with the human type than with the bovine type in the same dose. With the same type of bacillus the destruction is more complete in a given organ with a large dose than with a small dose. This is especially true of the spleen.

It is suggested that the virulence of a certain type of mammalian bacillus for the rabbit is to some extent inversely related to the rate of its initial growth. The human type grows faster in the body of the rabbit and brings about an earlier altered body reaction and destruction of the organism than does the more slowly growing bovine bacillus.

The destruction in the various organs, after the altered body reaction has been brought about, is rapid at first and progresses more slowly as time proceeds. So that even six months after the intravenous infection of rabbits with small doses of human tubercle bacilli they have not yet completely disappeared from the lung and spleen, while they can no longer be isolated from the liver, bone marrow and kidney.

Some evidence is presented that this acquired resistance to tuberculosis in the various organs is an increment of the natural resistance of the same organs to the tubercle bacillus.

Discussion

(Dr. Paul Lewis, Princeton, N. J.) I am very much interested in the presentation of this subject, which is really extraordinarily difficult to approach. Dr. Lurie has done a great deal of work for which he deserves a great deal of credit, and has approached the matter as one who has more data. I wish to ask a question which he has not touched upon. I inoculated a series of rabbits with virulent bovine and human bacilli, and we examined the lungs particularly with great care microscopically, and the results seemed in a sense to be conclusive in that at each period up to the seventh day the number of bacilli found in the bovine and human were very difficult to discriminate, as Dr. Lurie has shown, but where Dr. Lurie's results seem to show that there is a rapid and progressive multiplication microscopically. On direct examination nothing such appears with the human strain. This bacillus disappears after 7, 8 or 10 days except possibly in foci which are not related to any general condition in the lung and

these also in some rabbits and not in others, whereas the bovine seems to develop as if it were on culture media. The human bacillus is ordinarily regarded as much easier to cultivate. It is possible that these observations are not so unharmonious because of the culture adaptability of one strain more than the other. Ability to grow is certainly not easy to harmonize with our previous observations and one has to be a little slow to accept it as a general thing. The conclusions are very interesting and he is to be commended on the systematic and painstaking way he has carried on the subject.

(Dr. Lurie, closing.) In regard to the first question: I wish to state that I have not as yet completed the study by any means, and that this is not a final statement, but a suggestion of the general nature of the facts. These studies will be continued by microscopic methods. I am not sure, but it appears that the isolation of tubercle bacilli by cultural methods does not necessarily mean that they will be found by microscopic technique. We shall come back to this.

The second question is a very pertinent one. It is more difficult to isolate bovine than human tubercle bacilli from infected tissues. However, I was conscious of this difference from the very first. As a matter of fact, from two suspensions of human and bovine tubercle bacilli each containing the same amount by weight, fewer colonies of bovine tubercle bacilli will be recovered than human. If, however, tissue suspensions are added to the bacillary emulsions, it seems that you would recover similar numbers from bovine and human. Still this is only a preliminary statement. But the point I wish to stress is not the actual numbers of tubercle bacilli recovered but the relationship between them. The relative rise is much slower in the bovine than in the human.

With B. C. G. some work has been done where I found essentially comparable results, namely, a still more rapid growth than the human type followed by a still more rapid destruction.

THE BLOOD IN HOG CHOLERA. Paul A. Lewis, Princeton, N. J.

(Abstract not received.)

THE INFLUENCE OF VITAMINE B AND SUBSTANCES CONTAINED IN BLOOD ON THE LAG PHASE OF TISSUE CULTURES OF THE CHICK SPLEEN. F. A. Hecker (by invitation), Ottumwa, Iowa.

Abstract. The ten-day chick spleen when planted in a mixture composed of one part of chicken plasma, one part of normal salt solution or Ringer's solution, and one part of potato broth, shows the growth of an eighteen-hour culture in this mixture is markedly increased, when compared to a control culture planted in one part of chicken plasma and one part of normal salt solution or Ringer's solution. In addition to the foregoing experiments, some of the substances contained in blood, namely urea, uric acid, etc., were added in definite amounts to the normal salt solution or Ringer solution and were mixed in the same volume proportionally as just described. No inhibitory influence on the growth of the cells could be detected when some of them were employed. This observation confirms the work of Burrows, namely, that high concentrations of Vitamine B induce active cell growth.

THE EFFECTS OF AMNIOTIC FLUID ON SEROUS SURFACES. Shields Warren, Boston, Mass.

Abstract. The chemical study of amniotic fluid indicates that it is a combination of transudate from the maternal blood and secretion from the fetus. One

apparent function of the amniotic fluid is to prevent adhesions between the fetus and membranes.

When amniotic fluid is added to the peritoneal cavity of animals which have been operated on so as to produce adhesions it prevents entirely their formation in nearly one-half the cases, and materially reduces the number of adhesions in others. In only a few cases has it been a complete failure. The controls have uniformly shown dense adhesions.

This action is apparently due to two factors: (1) shortening the time of oozing from injured surfaces; (2) the lubricating effect of the presence between eroded surfaces of a fluid which is not rapidly absorbed. There appear to be no ill effects from using the amniotic fluid of one species in animals of another species. Amniotic fluid does not delay wound healing nor affect the rate of growth of fibroblasts in tissue culture.

The sterility of amniotic fluid as such is difficult to maintain. Consequently, a method of alcoholic fractional precipitation has been developed which gives a concentrated fraction of amniotic fluid which is practically as efficacious as the original fluid. If anything, those animals receiving amniotic fluid have shown less shock and smoother convalescence than the controls. At present the concentrate is prepared from the amniotic fluid of the outer sac of the cow. No ill effects have been noted from its use either in animals or in the limited number of human cases in which it has been tried.

Experiments are under way at the present time to determine the efficacy of amniotic fluid concentrate in joint cavities, the pericardial and pleural cavities, and the conjunctival sac. It may be of value in relieving the symptoms of fibrinous pleuritis or pericarditis.

The amniotic fluid concentrate as prepared at present contains a small amount of mercurial antiseptic, and it is to be hoped that in cases of peritonitis this fluid may be of advantage when combined with a larger amount of antiseptic. It has definite advantages over other substances which have been tried for the purpose of preventing formation of adhesions in that it does not induce a foreign body reaction, it does not act through digestion or destruction of tissue, and it does not interfere in any way, so far as can be determined, with normal function.

INTRA- AND SUPRAVITAL STUDIES OF FIXED AND CIRCULATING CELLS IN PERITONITIS. Bernhard Steinberg, Toledo, Ohio.

Abstract. Peritonitis was produced by intraperitoneal injection of colon bacilli suspended in gum tragacanth and by intraperitoneal injection of fecal material. Leucocyte counts were taken prior to the production of peritonitis. Following the production of the infection, counts were made at intervals of one-half and one hour. It was found that within two to three hours after the onset of infection, the leucocyte count in the peripheral blood dropped to one-half or one-third of the pre-infection number. Within six to seven hours, the number of leucocytes returned to the level prior to the production of the peritonitis. Following this period, the number of leucocytes rapidly increased and a marked leucocytosis occurred. This part of the experimental work showed that following the onset of the peritoneal infection, there was a marked drop in the leucocytes in the peripheral blood. The leucocytosis observed in infections apparently does not ensue for nine to ten hours after the onset.

Studies of the peritoneal smears during the course of acute peritonitis showed that the exudation of free cells occurs in non-immunized animals approximately three hours following the onset of the infection. The appearance of the free

cells was shortly after the drop of the leucocytes in the peripheral blood. The wandering cells (those found in the general circulation) were in predominance in the peritoneal exudate during the first five to six hours in the course of the peritonitis. Coincident with the rise of leucocytes in the peripheral blood, monocytes predominated in the peritoneal fluid. The monocytes were differentiated by the supravital staining with neutral red. Following the appearance of monocytes in the peritoneal exudate a small number were found in the peripheral blood. In immunized animals, the peritoneal exudate showed appearance of cells within one hour of onset of infection. Many clasmotocytes were found in the exudate. Bacteria in immune animals were rapidly clumped and phagocytized by monocytes and polymorphonuclears. Non-immune animals did not show clumping of bacteria.

Study of tissue at approximately two-hour intervals during the course of peritonitis showed in non-immune animals a slow cellular response. In those animals, the mononuclears predominated in the first several hours. The first reaction of the peritoneal tissue was distension and congestion of capillaries and smaller blood vessels. Following this was the appearance of polymorphonuclears within blood vessels. Within seven hours after the onset of peritonitis a number of polymorphonuclears and approximately an equal number of mononuclears were seen in the tissue. In the immune animals, the reaction was predominately polymorphonuclears.

Immune animals showed clasmotocytes in the peritoneal exudate. Apparently, the immunity in the animal was coincident with the appearance of those cells. Clasmotocytes were not found in non-immune animals. They were found, however, in small numbers in animals which were recovering from the infection. Immune animals show a more rapid and a greater cellular reaction both in the tissue and in the exudate.

Discussion

(Dr. H. L. Jaffe, New York City.) There are, as we know, difficulties in the process of staining cells. If you permit them to stand under the microscope for a period of time, because of the changes that take place in these cells or as the result of injury the type of granule varies considerably. So I feel that Dr. Steinberg should take a little caution in interpreting the character of cell he finds in the peritoneal exudate, because certainly a great number of these cells are injured at the time he stains them supravitaly.

(Dr. Steinberg.) I did not have time to present many of the methods employed in the experiments. The cells were examined immediately after removal from the peritoneal cavity, as a rule within one minute.

(Dr. Jaffe.) I meant injured at the time of removal.

(Dr. Steinberg, closing) If Dr. Jaffe means that the cells were already injured in the peritoneal cavity prior to removal, that is very probable, but it was possible to differentiate the types of cells, even though injury is to be admitted. I appreciate that injured cells may show altered capacity to dye intake but again we are dealing with constant factors; it is difficult to assume that in repeated experiments, properly controlled, only certain types of cells were injured and others exposed to the same conditions remained uninjured.

CELLULAR RESPONSE IN RABBITS TO REPEATED INJECTIONS OF NON-HEMOLYTIC STREPTOCOCCI. Robert N. Nye, Frederic Parker Jr., and (by invitation) David Seegal, Boston, Mass.

(Abstract not received.)

THE RHEUMATIC NODULE. B. J. Clawson, Minneapolis, Minn.

(*Abstract not received.*)

THE CERVIX UTERI AS A FOCUS OF INFECTION IN CHRONIC ARTHRITIS. L. W. Famulener and (by invitation) Frederick J. Matthews, New York City.

(*Abstract not received.*)

EARLY VISCERAL LESIONS OF TULAREMIA. Walter M. Simpson, Dayton, Ohio.

Abstract. A complete autopsy on the most rapidly fatal case of tularemia of which there is any record (five days) afforded an opportunity to study the histopathological characteristics of the early lesions of this disease. The increasing importance of this disease, both clinically and pathologically, is manifested by the fact that the writer unearthed forty-nine cases of this disease in Dayton, Ohio, during a brief period of four months.

The axillary and epitrochlear lymph nodes showed multiple recent areas of focal necrosis, miliary and submiliary in size. Surrounding the focal necroses there was early fibroblastic and epithelioid proliferation with a remarkable hyperplasia of the reticulo-endotheliocytes. The granulation tissue was entirely devoid of new-formed capillaries. Giant cells of the Langhans type, which have been described in practically every case previously reported, were absent, although some of the histiocytes had fused, forming a kind of symplasm, probably representing early giant cell formation. The normal architecture of the lymph nodes was destroyed.

The spleen showed similar focal necroses involving by far the greater part of the spleen. The diffuse reticulo-endothelial hyperplasia was more marked in the spleen.

The hepatic lesions varied markedly from the lymph node and splenic lesions. There was practically no caseous necrosis, the lesions being entirely made up of foci of reticulo-endothelial hyperplasia. In the right lung were two cherry-sized areas of caseous necrosis with epithelioid and fibroblastic proliferation and polymorphonuclear infiltration at the periphery.

The lesions described herewith should be classed with the infective granulomata, and are obviously earlier lesions of the disease than have been described heretofore.

Discussion

(Dr. H. H. Permar, Pittsburgh.) The lesions shown here by Dr. Simpson strike me as the most acute possible type of granuloma. I remember some subcutaneous lesions in a case of a much more chronic type in which we had marked hyperplasia of the vascular endothelium, giant cells, and all the features of a granulomatous reaction. The necrotic areas, it seems to me, looking at the lantern slides, show a certain number of polymorphonuclears among the necrotic tissue. I would like to know if that is correct. We found numbers of them in our sections in the same locations.

(Dr. E. R. LeCount, Chicago.) I should simply like to place on record that Dr. S. John House of Nashville, Tenn., showed me during the past winter microscopic preparations of human tissues with this disease and that we discussed the similarity the lesions possess to bubonic plague and others of the so-called hemorrhagic septicemia group. We also discussed the hazards of postmortem examinations of persons who died from these diseases. I believe they are very considerable, probably more than the average streptococcus infection. I have

no doubt the necroses shown us here in the lantern pictures contain a large amount of fibrin.

(Dr. G. R. Callender, Washington.) I would like to congratulate Dr. Simpson on having the earliest case of tularemia reported. Lesions of the type he has shown cannot be differentiated from the focal necroses of other septicemias. Those he has shown today approach the characteristic pathology seen in the guinea pig, and in some instances the pathology seen in the mouse. Even in this early lesion there is hyperplasia of the reticular cells. I believe those lesions are all too early to give rise to the response of giant cell formation. One thing about those focal necroses: a section through the outside of an area will show almost a pure endothelial proliferation, but if you make serial sections you will usually find that there is a beginning necrosis even in relatively small lesions.

(Dr. Francis, Washington.) Dr. Simpson has done something that is as fine as could be done. He has studied a number of cases in Dayton, Ohio, for the last two years. He diagnosed his cases by examining the blood and found the organism in the blood.

(Dr. Simpson, closing.) In many of the foci of the liver, we did make serial sections of these lesions and there was no extensive caseation present. As regards the danger of acquiring the infection from autopsies, it is, of course, very great. Twenty investigators have been victims of laboratory infections. We autopsied more than 100 animals during the course of this study, but we employed extraordinary precautions throughout. Nobody in our laboratory acquired the disease.

STUDIES IN THE PATHOLOGY OF SCHISTOSOMIASIS IN PORTO RICO (*S. Mansoni*).

Robert A. Lambert, San Juan, Porto Rico.

(*Abstract not received*)

UNUSUAL TUMOR OF THE KIDNEY. Alfred Plaut, New York City.

Abstract. White female, 59 years old. Removal of kidney stone, formation of a sinus; swelling in scar three months after operation. Persistence of sinus, increase of swelling; pyelogram indicates pyonephrosis. Nephrectomy one year after removal of stone. Gross specimen from outside looks like pyonephrosis. The calices are much dilated, no kidney tissue found, the outline of kidney is fairly normal. The calices are filled with thin brown fluid; much gelatinous material covers the inner surfaces, partly contained in small cysts. Innumerable small papillary protrusions cover the greater part of the calices. Similar papillae are seen in the ureter. The ureter is in open communication with the calices. The microscopic picture is mostly that of mucosa of large intestine. There are typical mucus producing glands, a loose propria, some lymph follicles and smooth muscle. An enormous papillary overgrowth has led to formation of thick masses of mucin in which many disintegrating cells are included. The mucin becomes red in the mucicarmine stain. Under these intestine-like glands other ducts are seen with low cuboidal clear epithelium and non-mucinous hyaline-like contents; they look similar to kidney tubules and they are situated in renal blastema as one sees it in embryonal kidney tumors of children. There are many abortive tubules and irregular small agglomerations of cells similar to the epithelium of the tubules. No striated muscle found. The ureter is lined by what appears as mucosa of large intestine. No renal blastema seen in the ureter.

This tumor cannot be classified together with any reported so far. It belongs halfway between the embryonal adenomyosarcoma of kidney and the intestine-like more or less tumorous changes which develop in the depth of the everted urinary bladder. Its similarity to large intestine is still more marked. It is a hamartoblastoma, probably multicentric or even diffuse in pelvis of kidney and ureter. Considering the rarity of such tumors and the frequency of kidney stones one cannot ascribe to the stone an important role in the genesis of this tumor. Embryonal kidney tumors have been observed repeatedly in the adult. The prognosis is doubtful especially because ureter with intestine-like epithelium has remained in the patient.

Discussion

(Dr. E. T. Bell, Minneapolis.) Out of 100 renal tumors from adults I have found two adenosarcomas of the infantile type, but these were unlike Dr. Plaut's cases in that they did not show mucin. The presence of mucin is difficult to explain, and in this respect these tumors differ from adenosarcomas previously described.

(Dr. Plaut, closing.) As to the presence of mucin I would like to make the general statement that I think the importance of mucin has been over-estimated. Often glandular structures coming from peritoneum might form mucin. I have seen mucin form in Fallopian tubes. I might add that there was no gelatinous transformation of connective tissue. Connective tissue was widely destroyed by gelatinous material.

SKELETAL METASTASES IN MALIGNANT TUMORS OF THE KIDNEY. Isaac Levin, New York City.

(Abstract not received.)

STUDY OF ARTERIES IN NON-SUPPURATIVE DISEASES OF THE KIDNEYS. N. W. Barker (by invitation), Rochester, Minn.

Abstract. The celluloid corrosion method has been used in a study of the course, relations, distribution and patency of the arterial system in human kidneys. A demonstration will be given of the gross arterial trees and photomicrographs of their terminal branches in some cases of non-suppurative renal disease as compared with those of normal kidneys.

Discussion

(Dr. Alfred Plaut, New York City.) How soon after removal of the organ or after death were these kidneys injected? Was the tissue still alive at the time of injection? Contraction of the blood vessels under the influence of the injection might change the picture.

(Dr. Barker, closing.) Of course it is impossible to obtain the kidney as you would like to have it. It is not possible to show functional changes in vessels. We obtain them as soon as we can and at postmortem they are washed out with ordinary tap water. Formerly normal saline was used. I believe it makes very little difference in the postmortem changes in the kidney. I think the kidneys were stained at least three hours after death, sometimes longer, five or six hours. All normal tissue, under normal conditions, shows no variation.

THE ARTERIAL SUPPLY OF THE KIDNEY IN NEPHRITIS AND ITS RELATION TO THE CLINICAL PICTURES. Saul A. Ritter (by invitation) and George Baehr, New York City.

Abstract. 1. Extensive vascular alterations similar to those of primary arteriolar disease develop as a regular phenomenon in cases of chronic diffuse nephritis (chronic Bright's disease) which survive the glomerulonephritis for a long enough period, and are not an accidental complication.

2. It is not alone the primary diffuse parenchymal disease, as has been generally thought, but rather this secondary vascular alteration which is responsible for the sclerosis and contraction of the secondary contracted kidney. Primary and secondary contracted kidneys in this respect are pathogenetically identical.

3. The clinical picture of arterial hypertension terminating finally in dry uremia may be associated with either the primary or the secondary contracted kidney. In our experience, the secondary contracted kidney is in fact much more commonly found at postmortem as the cause of this picture.

Injection studies upon the renal arterial system explain one of the apparent discrepancies between clinical findings and pathological types of chronic nephritis, and demonstrate that complete harmony exists if the significance of the essential pathological process is recognized.

NEPHROSIS WITH GLOMERULONEPHRITIS (CASE REPORT). Margaret Warwick, St. Paul, Minn.

Abstract. A case report of a woman 34 years of age who had scarlet fever at the age of 18 and diphtheria at the age of 32. Her present illness was of six months duration and consisted of malaise, drowsiness, edema, ascites and high blood pressure, with casts, albumin and low specific gravity in the urine, and a secondary anemia. Her creatinin went up to 7.2 and urea nitrogen up to 47, but her eye-grounds remained normal.

The autopsy showed a complete absence of the left kidney, although a small, patent ureter and pelvis were present. The right kidney weighed 300 gm., the capsule was loose, the cortex thick and pale, but the pelvis was normal. Microscopic sections showed lesions affecting both the glomeruli and the tubules. In some of the glomeruli there was a proliferation with the formation of new connective tissue and the fusion of the tuft with Bowman's capsule; in many others there was a marked infiltration with polymorphonuclears and a swelling of the endothelium of the tuft capillaries, while yet others remained normal in appearance. The tubules all showed a marked atrophy consisting of a decrease in size and a lining of very low or flat epithelium. The tubular injury seemed to be out of all proportion to the glomerular injury since there were some normal glomeruli, and no normal tubules could be found. Furthermore, the lesions in many of the glomeruli do not seem to be severe enough to account for the extensive tubular atrophy. Therefore, lesions seem to be affecting the tubules and glomeruli independently instead of the tubular injury being secondary to the glomerular injury. The only possible classification seems to be the so-called mixed form with the presence of both a nephritis and a nephrosis.

Discussion

(Dr. B. H. Lucké, Philadelphia.) It seems to me that all these cases present the usual picture of subacute glomerulonephritis.

(Dr. G. Baehr, New York City.) I would like to agree with the previous speaker and call attention to the fact that the clinical diagnosis of nephrosis is

being made altogether too frequently. Every case of glomerulonephritis has a certain nephrotic element. At the Mt. Sinai Hospital in New York, where we have studied nephrosis for a long time, we have only once encountered a pure nephrosis in an adult at autopsy. Every other case clinically diagnosed nephrosis has at autopsy presented characteristic evidences of glomerulonephritis. The one time nephrosis occurred in an adult was in a syphilitic. In children we do see pure nephrosis not infrequently. In my experience they are usually superimposed upon a chronic pneumococcus infection in the upper respiratory tract. Eventually most of these children die of a pneumococcus infection of their ascites.

(Dr. K. M. Lynch, Charleston, S. C.) It seems to me that there are two separate lesions in the kidney. I agree with the other speakers that the term nephrosis is not applicable. It seems to me there is an older interstitial sclerosis and an apparently recent glomerulonephritis. The dilatation of tubules may be related to the absence of the other kidney. Not uncommonly I see true chronic diffuse interstitial nephritis. I see no reason why this condition with superimposed subacute glomerulonephritis could not explain this kidney.

(Dr. Warwick, closing.) I feel justified in the case report because of the discussion brought out. I think the discussion of the term "nephrosis" has been very well answered. Perhaps my title on the program was not clear, I think it should not have been stated as it was. I meant to say I had lesions of both tubules and glomeruli, so I called it nephrosis with glomerulonephritis, which is not as accurate as it might be. With these kidneys we feel very definitely that the lesions of the tubules were out of proportion to the lesions in the glomeruli and the absence of the other kidney may be the cause of it.

EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED BY THE TUBERCLE BACILLUS.

Leone McGregor (by invitation), Rochester, Minn.

Abstract. Calmette's attenuated bovine tubercle bacillus (BCG) was introduced intra-arterially into the kidneys of rabbits.

The injections were made into the superior mesenteric artery against the blood stream, after first applying two clamps, one on the aorta below the left renal artery, the other on the superior mesenteric artery just distal to the point of injection. In this way most of the BCG emulsion was driven through the renal arteries. The kidneys of 10 rabbits were removed at regular intervals so that stages from one to twenty days could be studied.

Sections of these kidneys show exudative and marked proliferative changes in a large proportion of the glomeruli, with obliteration of their capillary circulation and beginning tubular atrophy.

Discussion

(Dr. E. R. Long, Chicago.) This is of special interest to me in connection with the studies I have just made myself, to be reported in the next paper. I would like to ask Dr. Bell if more than one injection was made. If so, the phenomenon of sensitization might have entered. It is interesting to recall that the tubercle bacillus is a rather complex structure in which more than one kind of chemical substance is present and important. There are the proteins, to which the host becomes specifically sensitive, and there are the lipoids which act as a foreign body. It would be interesting, therefore, to know if there was any difference between injecting the whole bacterial cell and injecting simply the lipid. I have made intracardiac injections, but never produced as severe a picture as

that shown and did not reach anything like sixty per cent of the glomeruli. I have been accustomed to interpret the changes occurring in the tubules when the glomeruli are blocked off as atrophy from anemia and therefore malnutrition, rather than atrophy from disuse. Is not that the case?

(Dr. G. Bachr, New York City.) Years ago we attempted to produce glomerular lesions by injection of streptococci into the renal artery and found it impossible to produce bacterial emboli in any very large percentage of the glomeruli. The reason is obvious since Richards has demonstrated that all of the glomeruli are not working all the time. You therefore cannot expect by any intravascular injection to damage all the capillaries in all the glomeruli or in any very large percentage of them.

(Dr. Long.) Do you think that would be the case if you made a perfusion with a special apparatus which might be run over half an hour? All the glomeruli are not in use at one time. There are cycles, a particular group carrying the load for 10 to 15 minutes, and then closing for a period. Do you not suppose you might get all the glomeruli if the injection were properly prolonged?

(Dr. E. T. Bell, Minneapolis.) The needle was inserted into the superior mesenteric artery and two injections were given with an interval of about 15 minutes. It is not desirable to occlude all the glomeruli since this results in uremia. We have used only Calmette's attenuated bovine culture, BCG. This is an ideal organism to produce proliferative lesions.

EXPERIMENTAL SUBACUTE GLOMERULONEPHRITIS PRODUCED BY INTRARENAL TUBERCULIN REACTIONS. Esmond R. Long and (by invitation) Lucy Finner and Paul J. Patchen, Chicago, Ill.

Abstract. Acute and subacute forms of glomerulonephritis were produced by arterial perfusion of the kidneys of tuberculous swine under ether anesthesia with a finely flocculent suspension of purified tuberculin protein. Similar perfusion of the kidneys of normal swine resulted in no appreciable change. The exudation within the glomeruli was therefore interpreted as a true tuberculin reaction, analogous to that produced by the injection of tuberculin into the skin or testis of a tuberculous animal. Epithelial and endothelial organization of the glomerular exudate resulted within two months in glomerular changes similar to those seen in some forms of subacute glomerulonephritis in man. In the course of ten months after the operation almost complete recovery occurred, only a small amount of glomerular scarring persisting. The course of anatomical change was observed by biopsy, nephrectomy or killing of some of the animals in the series. During the subacute stage of the process, after bilateral perfusion, the non-protein and urea nitrogen of the blood were more than doubled. With anatomical recovery the blood chemistry returned to normal.

Discussion

(Dr. P. O'Hare, Boston.) I would like to ask the speaker if he feels that this is a sensitization process. If it is such, why does he not get the same reaction with the tuberculin alone?

(Dr. C. W. Duval, New Orleans.) I would like to ask Dr. Long and his co-workers if they found it necessary to make the injections of tuberculin directly into the renal vessels; could not the same lesions be produced by giving the tuberculin subcutaneously or intracardially?

(Dr. E. T. Bell, Minneapolis.) Dr. Long has shown us a very good example of focal glomerulonephritis. This is evidently a localized tuberculin reaction in

the kidney and corresponds to the skin reaction in a sensitive animal. The question is whether or not this sensitization plays a rôle in human nephritis.

(Dr. S. R. Haythorn, Pittsburgh.) Even though we can get a glomerulonephritis experimentally in this way it is difficult to see what happens in the tuberculous subject. Unless the kidney is able to protect itself in some way every tuberculous patient should develop a glomerulonephritis each time a caseating lesion releases tuberculous toxin. All tuberculous patients then, should die early of glomerulonephritis. It is well known that tuberculous cattle may be given a desensitizing dose of tuberculin which will protect them from a positive tuberculin test for several weeks. This procedure is known as "plugging." A similar mechanism may apply in the tuberculous patient since one rarely finds acute glomerular lesions. A few years ago I reported a case of a tuberculous aneurysm of the common iliac artery and in the glomeruli of the kidney there were masses of tubercle bacilli. Most of the glomeruli showed no reactions. There were capillary loops blocked with tubercle bacilli in which no reaction of any kind was present. If a tuberculin reaction occurs in a sensitized kidney in the human it must be a very temporary thing or else the kidney must protect itself in some way, perhaps by "plugging" as the expression is applied to cattle.

(Dr. G. Baehr, New York City.) The rôle which sensitization plays in the production of human glomerulonephritis is best brought out possibly in subacute bacterial endocarditis due to the streptococcus viridans. In our observations about 17 per cent of patients with subacute streptococcus endocarditis develop a complicating acute diffuse glomerulonephritis. This acute glomerulonephritis has no relation to the embolic glomerular lesions found in this disease. The true acute glomerulonephritis seems to occur only in those patients who have shown a tendency to kill off their bacteria. With few exceptions the patients with subacute bacterial endocarditis who died of an acute glomerulonephritis or its sequel, chronic diffuse nephritis, were all in the bacteria-free stage of the disease as described by Libman, or were becoming bacteria-free. Patients with subacute streptococcus endocarditis, in whom the blood cultures are persistently positive, and who show no such tendency to become bacteria-free, do not as a rule develop an intercurrent diffuse glomerulonephritis.

(Dr. Long, closing.) In reply to Dr. Duval's question, as to whether subcutaneous injections would not work just as well as intrarenal injections, the answer is no. We found it necessary to make these into the renal artery. Subcutaneous and even intravenous injections were not sufficient.

With regard to Dr. Bell's remarks: I feel myself that probably the lesions one sees in the kidneys in subacute bacterial endocarditis may be partly on this allergic basis. It is certainly probable that if a patient has this disease for some months the body becomes sensitized. The interaction between the sensitized tissue of the glomeruli and the sensitizing bacteria might well result in what we call subacute bacterial nephritis.

I am greatly interested in the work of Dr. Johnson, having developed the medium in which the bacteria for the work were grown. Colleagues associated with him have worked chiefly with the injection of these products, similar to the one we used here, in normal animals and also in normal cells, such as the ameba, and I believe they are dealing with a special type of foreign body reaction to substances differing in their individual primary toxicity. It is not the type of reaction we are concerned with here, which has to do with the reaction between specifically sensitized cells, and the antigen to which they are sensitive.

I think the answer to Dr. Ewing's question is that this work may not be so far removed from application to the problems in which we are interested as he supposes. There are other reactions taking place in the body, reactions we are all familiar with, that might be interpreted as tuberculin reactions. For instance, a tuberculous process spreads from the lung of a person infected with tuberculosis to the pleura; the result is an acute pleuritis, pleuritis with effusion, which many people believe is really a tuberculin reaction. Joint lesions in tuberculosis and tuberculous meningitis may likewise be truly responses of sensitized tissues to the substance to which they are sensitive, with the added element of morphological tuberculosis, of course, in all these instances.

There are probably many non-specific changes taking place in tuberculosis also. I admit it is still doubtful whether the kidney shares in this type of reaction. I cannot reconcile these results entirely with the facts to which Dr. Haythorn has called attention. A closer study of the facts is needed. To Dr. Bachr's question I might answer yes. We use a suspension in order to delay the speed of progress through the kidney. We have to put the reaction partly on an embolic basis, lasting a few minutes or more likely several hours.

MITOCHONDRIA IN EXPERIMENTAL ACUTE NEPHRITIS. Robert A. Moore (by invitation), Columbus, Ohio.

Abstract. An attempt has been made to determine the variations that mitochondria undergo in bichloride nephrosis and to correlate these findings with the degree of functional impairment of the cells involved. The mitochondria were best studied with the anilin fuchsin-methyl green stain (Cowdry's modification) but iron hematoxylin and Janus green B were also used. White rats were used and blood urea determinations were made on all animals just prior to death. After carefully eliminating all normal variations the following conclusions were reached. The mitochondria in the third part of the convoluted tubule are the first to show any variation. This variation comes after there has been renal insufficiency as evidenced by urea retention. In other words there is renal insufficiency before there are changes in the mitochondria.

These changes are a slight swelling and marked agglutination. Later the same changes take place in part 1 and 2 of the proximal convoluted tubules. On the basis of these findings, it is proposed that bichloride produces a nephropathy first by being adsorbed at the mitochondrial-cytoplasmic interface and later because of increasing concentration at this point the entire cell is killed.

EXPERIMENTAL STUDY OF ACQUIRED RESISTANCE OF THE RABBIT'S RENAL EPITHELIUM TO URANYL NITRATE. Warren C. Hunter (by invitation), Portland, Oregon.

Abstract. Although the ability of renal epithelium to regenerate after injury has been frequently demonstrated, little is known concerning the nature of the new cells. The purpose of this study was to determine whether regenerated kidney cells are as susceptible to injury by nephrotoxins as the original epithelium or become resistant or immune to such substances. The experimental work of Gil y Gil, the only previous investigator in this field, has been repeated and confirmed.

Preliminary experiments showed that 0.001 gm. of uranium subcutaneously or 0.0005 gm. intravenously, each representing one-half the lethal dose for rabbits, regularly kills normal proximal convoluted tubular epithelium. Complete regeneration occurs in two weeks. Differences in morphology and staining

readily distinguish the new epithelium from the original. Ample time for regeneration was allowed between the periodically increased injections. Only those animals surviving the last injection sufficiently long for fresh necrosis to appear in the persisting original tubular cells and less than the time required for new regeneration to begin are included in the series.

A high degree of acquired resistance to uranium is evidenced by lack of microscopic changes in the regenerated epithelium after giving 54 to 96 times the amount known to kill the original cells in the same tubules. Intravital staining indicated that the regenerated tubules were patent. Qualitative chemical tests and biological experiments proved that chronic uranium kidneys were able to excrete the metal in greater quantities than acutely damaged organs. The acquired resistance may be due either to the cells detoxicating the poison or to preventing its combination with their proteins.

In rabbits long-continued repeated administration of uranium leads to marked chronic glomerulo-tubular lesions with secondary contraction.

CORRELATED GROSS, MICROSCOPIC AND CLINICAL OBSERVATIONS UPON 160
CONSECUTIVE PAIRS OF KIDNEYS. James E. Davis, Ann Arbor, Mich.

Abstract. Material: A series of 200 consecutive autopsied subjects who had been patients in four of Detroit's hospitals.

Plan of Study: The routine clinical records represent standard data obtained from all types of cases entered in the hospital as house patients. Eighty-five per cent of the total number were patients in the Receiving Hospital of the City of Detroit, and fifteen per cent were from Providence, St. Mary's, and Woman's Hospitals. It should be mentioned that a certain number of emergency cases included had insufficient data.

In addition to the general survey made at each autopsy by three members of the staff in Pathology and the writers, special routine studies were made of the kidneys, observations being directed to:

1. The thickness and adherence of the capsule.
2. The appearance of the cortex after the capsule had been stripped.
3. The relative proportions of the cortex and medulla seen on section.
4. The amount of peripelvic fat.
5. The presence or absence of striations in cortex and medulla.
6. The amount of apical blanching in the pyramids, and its degree.
7. The character of the pelvic mucosa.
8. The character of the superior part of the ureters.
9. The total weight of the organs stripped of fat.

Microscopic observations were made of two to four sections from each case, and attention was directed to changes in the arterioles, arteries, tubules, stroma and mucosa. In addition to diagnosing the presence of infection, degeneration, or both, an estimation was made of the degree of postmortem autolysis; also whether or not the blood pressure was raised; whether there was nitrogen retention, albuminuria, edema or drainage insufficiency. These observations were made without any knowledge of the clinical or gross conditions, and gradings were made by a second person who held the known clinical and gross data. This plan was used in order to evaluate, independently, the microscopic changes. The results obtained are of considerable value in proving the utility of certain routine observations and a classification, based quite strictly upon anatomical divisions of the kidney.

It was found advantageous to observe arteriolar changes in the glomeruli and their afferent and efferent vessels as the chief criterion of rise in blood pressure and nitrogen retention. Degeneration of the tubular units after excluding the effects of postmortem autolysis was used to indicate the effects of toxicity and infection. Stromal tissue increase was considered a factor decreasing efficiency in drainage. Albuminuria was diagnosed where active degeneration was present, and where active inflammatory reaction prevailed, accompanied by marked arteriolar reaction. Our practical experience proved that estimation of the presence of edema was most difficult. It is reasonable to expect this because the condition of the kidney is not solely responsible for the production of edema. However, correct estimation of the condition was made in sixty-five per cent of the cases.

If the additional information obtained from the gross study of the kidney removed as a surgical procedure be considered with the microscopic information, the correlation work will be enhanced. It is to be remembered that the microscopic and gross evaluations, when viewed solely from the clinical standpoint, may be considered at first thought impractical. However, it may be emphasized, that accurate and painstakingly acquired clinical data will point as surely to fixed structural changes, as will structural changes to the clinical diagnostic essentials.

The following classification was found most effective for correlation:

- Vascular (arterio and arteriolar) nephritis and nephrosis
- Tubular nephritis and nephrosis
- Stromal nephritis and nephrosis
- Focal nephritis (and focal pyonephritis) and nephrosis
- Ureteronephritis and pyoureteronephritis
- Perinephritis.

GENERAL DISCUSSION OF GLOMERULONEPHRITIS, NEPHROSIS AND RENAL ARTERIOSCLEROSIS. E. T. Bell, Minneapolis, Minn. (Appointed by the Council to act as reviewer).

Abstract. Classification of Nephropathies:

- I. Nephroses
 - (a) Chemical
 - (b) Infectious
 - (c) Pregnancy
 - (d) Lipoid
 - (e) Amyloid
- II. Glomerulonephritis
 - (a) Focal
 - 1. Embolic
 - 2. Benign hemorrhagic
 - (b) Diffuse
 - 1. Acute
 - 2. Subacute
 - 3. Chronic
 - (a) With lipoid nephrosis
- III. Arteriosclerotic Kidney
 - (a) Without renal insufficiency
 - (b) With renal insufficiency
 - 1. Slowly developing type
 - 2. Acute fulminating type

IV. Exudative Interstitial Nephritis

(a) Acute interstitial nephritis

(b) Pyelonephritis

1. Hematogenous

2. Urinogenous

In classifying the nephropathies, pathologists should keep in mind the functional as well as the structural changes in the kidneys. In so far as possible, we should adjust our nomenclature to correspond with recognizable clinical entities. An ideal classification based entirely on etiology is not possible at present, since the same organisms (*viz.*, streptococci and pneumococci) seem to cause certain forms of nephrosis as well as glomerulonephritis and exudative interstitial nephritis. The symptoms and clinical course of a nephropathy are determined by the following circumstances: (a) the histologic elements affected, *i.e.*, glomeruli, tubules, arteries, interstitial tissues; (b) the *character* of the process, *i. e.*, exudative, proliferative, degenerative; and (c) the *degree* of the injury. The foregoing classification is based partly upon the histologic elements affected and partly upon the character of the lesion.

I. NEPHROSES

Nephropathies of a purely degenerative character are called nephroses. The lesions are chiefly confined to the tubules but often the glomeruli and rarely the arterioles show degenerative changes. Glomerular lesions of a degenerative character are classified as nephroses. The alterative changes are cloudy swelling and fatty metamorphosis in the milder forms and hyaline granular degeneration and necrosis in the severe types.

(a) *Chemical Nephroses.* The representative of this group which is of greatest clinical interest is the corrosive sublimate kidney. This is characterized clinically by oliguria and anuria, moderate albuminuria, slight edema and progressive renal insufficiency. Associated with the anuric stage, there is often a moderate hypertension. Anatomically there is marked tubular degeneration and necrosis, often with calcification of the necrotic tubules.

The experimental nephropathies obtained with uranium nitrate, potassium bichromate, and so forth, belong with the nephroses, since except for an occasional epithelial crescent, the only glomerular lesions obtained are of alterative character. This is true even of the experiments of Wiesel and Hess, who obtained extensive necrosis of the glomeruli. Purely alterative glomerular lesions should not be called glomerulonephritis.

(b) *Infectious Nephroses.* Nearly all severe infectious diseases or infections are accompanied by albuminuria of varying degree; and at postmortem in such cases the kidneys show cloudy swelling. In rare instances, there is anuria clinically, and a widespread necrosis of tubules and glomeruli is found at postmortem. Necrotic nephroses of this type have been described in diphtheria, cholera and severe septicemia.

The infectious nephroses show gradual transitions to acute glomerulonephritis. There is often a slight swelling of the glomerular endothelium, and when this change becomes sufficiently pronounced it is called glomerulonephritis. Often the decision between infectious nephrosis and acute glomerulonephritis is arbitrary. The pathogenesis of glomerulonephritis is best studied in the infectious nephroses.

The infectious nephroses are rarely cases of renal disease in the clinical sense, since they are definitely overshadowed by the major illness.

(c) *Nephrosis of Pregnancy*. The toxemia of the latter part of pregnancy (called eclampsia when convulsions occur) is characterized by albuminuria, hypertension and edema. There is often oliguria and there may be anuria; but usually renal function is fair. At postmortem the kidneys are found cloudy and greatly swollen. There is a moderate amount of lipoid in the tubules and glomeruli. Occasionally there is moderate or even extensive tubular necrosis. In five of ten cases which I have studied microscopically, there was a marked swelling of the glomerular endothelium with definite narrowing of the capillaries. The enlargement of the glomeruli is only slight and I have not proved that proliferation occurs; but the effect on the circulation is the same as that of a glomerulitis.

Aside from the glomerular changes, which are not constant, the anatomic picture corresponds with that of a lipoid nephrosis. A few chemical studies that have been made do not show clearly whether the chemical changes in the blood correspond with those observed in pure lipoid nephrosis.

(d) *Lipoid Nephrosis*. This is the same as the "genuine nephrosis" of Volhard and Fahr, the "chronic nephrosis" of Epstein and the "lipoid nephrosis" of Munk. Pure cases of lipoid nephrosis are characterized clinically by severe albuminuria and edema, and the absence of hypertension and renal insufficiency. There is an increase of blood cholesterol and a decrease of the plasma proteins, especially of the albumin, so that the albumin-globulin ratio becomes reversed. Lipoids are found in the urine and the edema fluids are low in protein. It is a chronic disease that usually ends fatally but sometimes in recovery.

Certain cases of chronic glomerulonephritis show edema, albuminuria, increased blood cholesterol and decreased serum proteins. These may be distinguished from a pure lipoid nephrosis whenever hypertension or renal insufficiency is present; but since both of these signs are occasionally absent in glomerulonephritis, we must admit that the final diagnosis of pure lipoid nephrosis must be made postmortem. Notwithstanding the difficulty in establishing the diagnosis clinically, there can be no doubt that cases of pure lipoid nephrosis do occur.

The kidneys are large and cloudy and there is abundant lipoid in the tubules. The glomeruli are normal.

We have no definite information as to the etiology of pure lipoid nephroses. It is known that the partially obstructed glomeruli of chronic glomerulonephritis are often associated with a large tubule full of lipoid; but there is no anatomic glomerular damage in pure lipoid nephrosis.

(e) *Amyloid Nephrosis*. This renal lesion is usually secondary to chronic suppuration and is overshadowed clinically by the infection. There is usually marked albuminuria, and edema is frequent. Sometimes the blood shows the changes characteristic of a lipoid nephrosis. The anatomic changes are amyloid infiltration of glomeruli with hyaline, granular and fatty degeneration of the tubules. The glomeruli often become completely occluded so that the tubules atrophy and a contracted kidney results. Cases that end with renal insufficiency often show hypertension also. One case of amyloid kidney which I studied developed acutely following a streptococcic suppuration and in association with an acute glomerulonephritis. The amyloid kidney is related to glomerulonephritis.

II. GLOMERULONEPHRITIS

(a) *Focal Glomerulonephritis*. When only a part of the glomeruli show inflammation, the lesion is called focal glomerulonephritis.

1. *Embolic Glomerulonephritis*. A well recognized nephritis of this type is Löhlein's embolic form, which develops in association with bacterial endocarditis. Clinically, it appears as a complication of bacterial endocarditis and is often recognizable by the appearance of hematuria in this form of cardiac disease. Microscopically, the typical lesions are areas of necrosis involving one or more lobules in a glomerulus. These lesions are believed to result from small infected emboli from vegetations on the valves of the heart. Occasionally lesions of a proliferative character are noted which involve a part or all of the glomerulus. Glomerular abscesses are occasionally observed. Diffuse glomerulonephritis is almost as frequent as the embolic type as a complication of bacterial endocarditis.

2. *Benign Hemorrhagic nephritis*. In children and young adults, one often sees hematuria which lasts from a few days to several weeks. The hematuria follows some infection such as a tonsillitis and is not accompanied by edema, hypertension or renal insufficiency. Fahr calls this condition focal non-embolic or excretory glomerulonephritis; but Baehr's suggestion, "benign hemorrhagic nephritis" seems to be more descriptive of the disease. No pathologic studies are available, but a study of hematurias associated with fatal infections suggests that the underlying anatomic change is rupture of the wall of glomerular capillaries. It is possible that the disease is a very mild diffuse glomerulonephritis, since many cases of hemorrhagic nephritis are known to be of this type. When anatomic studies become available it may be necessary to put this disease in some other group.

(b) *Diffuse Glomerulonephritis*. In well developed cases of this disease, nearly all the glomeruli show inflammatory changes. Clinically the characteristic features are albuminuria with casts and often with blood, edema and hypertension. Edema varies greatly in different cases and also from time to time in the same individual. Hypertension may be absent in mild acute cases and in certain chronic cases.

The glomerular lesions are of three histologic types: exudative, proliferative and extracapillary.

(1) The exudative lesion consists in the accumulation of large numbers of polymorphonuclear leucocytes within the glomerular capillaries. The leucocytes may pass out into the capsular space and into the tubules. Pure exudative glomerulitis is uncommon; there is usually an associated proliferation of the endothelium.

(2) Proliferative glomerulitis is the most common type. It consists in swelling and proliferation of the glomerular endothelium with enlargement of the glomerulus and narrowing or closure of the capillaries. Polymorphonuclear leucocytes are often imprisoned in the closed capillaries.

(3) The extracapillary type consists in proliferation of the cells of the parietal layer of the capsular space with the formation of an epithelial crescent which compresses the glomerulus.

Pure exudative lesions probably cause only temporary occlusion of the glomerular capillaries, but proliferative lesions cause permanent narrowing or complete closure. When the blood can no longer go through the glomerulus, secretion of urine ceases and the tubule undergoes disuse atrophy. By the time that the glomerulus has become hyaline, the associated tubule has disappeared.

Atrophied tubules are replaced to some extent by connective tissue — an appearance which is responsible for the term “chronic interstitial nephritis” which is often applied to the contracted kidney of chronic glomerulonephritis.

When the glomerular capillaries are narrowed but not completely closed, the associated tubule does not atrophy but enlarges, and the cells accumulate lipoid droplets. The large kidneys of this type were formerly called “chronic parenchymatous nephritis.” This form of chronic glomerulonephritis is characterized by severe albuminuria and edema and often shows the changes in the blood characteristic of lipoid nephrosis. Hypertension is sometimes absent.

III. ARTERIOSCLEROTIC KIDNEY

(a) *Without Renal Insufficiency.* Approximately 90 per cent of persons who have primary hypertension do not show renal insufficiency. The kidneys show varying degrees of involvement of the arteries and arterioles but there is not sufficient atrophy to cause a definite decrease of renal function. The parenchyma does not undergo atrophy until the artery or arteriole is markedly narrowed. There are all degrees of atrophy in the kidneys of persons who die of primary hypertension, from normal kidneys to small contracted kidneys. Only about 10 per cent show a sufficient degree of atrophy to justify the term “primary contracted kidney.” In hypertension without renal insufficiency, death is due to congestive heart failure, coronary sclerosis or apoplexy.

(b) *With Renal Insufficiency.*

1. The usual case of this group shows clinically a slowly developing uremia. The kidneys are small and show granular surfaces — “primary contracted kidney.” The atrophy is due to narrowing and closure of both small arteries and arterioles.

2. Certain persons who have had hypertension for a long time without any signs of serious renal involvement develop uremia rather acutely, often as a result of some infection. Occasionally, an acute hypertension with uremia develops in a person who was previously normal. Such cases are sometimes called “malignant” hypertension. The kidneys in these cases may not be contracted, but microscopically a widespread necrosis of arterioles is found.

IV. EXUDATIVE INTERSTITIAL NEPHRITIS

(*Not discussed at meeting*)

THE HISTOPATHOLOGY AND BACTERIOLOGY OF EXPERIMENTAL POLIOMYELITIS IN THE MONKEY. E. C. Rosenow, Rochester, Minn.

Abstract. Cultures of brain and cord of 142 monkeys that developed poliomyelitis following inoculation of virus, by methods similar to those previously described, yielded greening streptococci in ninety-two or 65 per cent, indifferent streptococci in 8 per cent, hemolytic streptococci in 6 per cent, staphylococci in 46 per cent and bacilli in 25 per cent. Greening streptococci were isolated in 80 per cent of eighty-seven monkeys that were cultured in from one to three days after onset of paralysis, in 60 per cent of twenty cultured four to six days after onset of paralysis, in 37 per cent of sixteen cultured seven to ten days after onset of paralysis, and in 26 per cent of nineteen cultured eleven days or longer after onset of paralysis.

Similar cultures from the central nervous system of fifty-six monkeys inoculated intracerebrally with the greening streptococcus yielded this organism in

52 per cent, indifferent streptococci in 9 per cent, hemolytic streptococci in 12 per cent, staphylococci in 27 per cent and bacilli in 21 per cent. Greening streptococci were isolated in 60 per cent of fifteen monkeys cultured from one to three days after inoculation, 69 per cent of sixteen monkeys cultured four to six days after inoculation, 25 per cent of eight cultured from seven to ten days after inoculation and 41 per cent of seventeen cultured eleven days or longer after inoculation. Cultures from sixty-nine control monkeys, chiefly those that remained well following inoculations of virus and of the streptococcus and those that died of tuberculosis or colitis, yielded the greening streptococcus in 25 per cent, indifferent streptococci in 9 per cent, hemolytic streptococci in 4 per cent, staphylococci in 26 per cent and bacilli in 10 per cent. The agglutinating power of various batches of the poliomyelitis antistreptococcus serum over greening streptococci was tested in order to determine their immunologic condition. Ninety-two per cent of seventy-nine strains isolated from seventy-nine monkeys that developed poliomyelitis following inoculations of virus were agglutinated specifically by this serum. Ninety-two per cent of thirteen strains isolated from thirteen monkeys inoculated with the streptococcus obtained in cases of epidemic encephalitis and epidemic hiccup were agglutinated specifically in contrast to 13 per cent of fifteen strains isolated from fifteen control monkeys.

A microscopic study of sections of brain and cord was made of thirty monkeys that had acute poliomyelitis following inoculations of the virus and of twenty-five controls, mainly those that remained free or had doubtful symptoms following inoculations of virus, and those examined a long time after onset of symptoms and in which sections were free from poliomyelitic lesions. Of the former, all had typical lesions of poliomyelitis in varying degree. By the use of a modified Gram method of staining and diligent search, Gram-positive cocci and diplococci of varying size and shape, sometimes arranged in short chains and clumps of large and exceedingly small forms, were found in or adjacent to lesions in each of the thirty monkeys that had symptoms and lesions of poliomyelitis. In some instances similar organisms were found in direct smears of the cerebral and ventricular fluid and crushed material from the anterior horn of medulla and cord. The series included animals inoculated intracerebrally and intraspinaly with fresh and glycerolated emulsions of virus and filtered virus as well as animals that developed poliomyelitis following intravenous injection and after packing of the nose with gauze soaked in emulsions of virus. Diplococci were uniformly absent in sections and smears of control monkeys as well as in the normal tissues of the poliomyelitic animals remote from the lesions.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) May I ask a question from the depth of ignorance? In the first table it seems there were 85 per cent organisms other than the streptococcus viridans in the first set of animals and in the control set of animals with streptococcus viridans and not only were there no organisms recovered, but there was 69 per cent of other organisms. It would be interesting to know what Dr. Rosenow thinks the reason for their being there and also whether that does not change the etiological significance of the streptococcus viridans to a certain extent.

(Dr. Rosenow.) I did not quite get Dr. Krumbhaar's point. You say there were other organisms?

(Dr. Krumbhaar.) Yes.

(Dr. Rosenow.) You were adding all other types.

(Dr. Krumbhaar.) Are you not allowed to add them?

(Dr. Rosenow, closing.) Yes, you may add them but it would have no meaning. The exact significance of the isolation of the species of bacteria other than the greening streptococcus is not entirely clear. However, in view of the fact that considerable exposure to the air is unavoidable in obtaining pieces of brain and cord and in emulsifying these for culture, that the highest percentage consisted of staphylococci and bacilli, that so often they were found in only one or two of a large number of tubes inoculated, that they were isolated nearly as often in the control series as in poliomyelitic animals, and that these and the indifferent and hemolytic streptococci were not agglutinated by the serum of affected animals nor by the poliomyelitis hyperimmune horse serum, there is little doubt but that they were chiefly contaminations from air or passive invaders. Since greening streptococci were isolated in a far greater percentage of animals than any other species and in proportion to the earliness in the attack and since this is the only species that had antigenic effects as measured by the agglutinating power of the serum of inoculated animals, the isolation of other organisms in smaller numbers, over which the serum showed no agglutinating power, need not detract from the etiologic significance of the greening streptococcus.

STATISTICAL STUDY OF THE CAUSES OF DEATH IN 200 CASES OF COARCTATION OF THE AORTA (ADULT TYPE). Maude E. Abbott, Montreal, Canada.

Abstract. The findings in a personal case of complete obliteration of descending arch with impending rupture of the ascending aorta and with cerebral death in a boy of 14, are compared with 199 other cases in subjects over two years, in the literature, and a statistical study is presented showing the degree and character of the stenosis, relative frequency of a collateral circulation, cardiac hypertrophy, bicuspid aortic valve, acquired valvular lesions and acute infectious hypoplasia or dilatation of the aorta and of the various modes of death, in these 200 cases. The material reviewed consists of 136 cases abstracted by certain older writers on this subject, and 64 collected from the recent literature, the latter with the help of Dr. L. Minor Blackford of the Mayo Clinic.

Coarctation is commoner in the male than in the female sex, the proportion in this series being as 3 to 1, or 147 males to 48 females. Its symptomatology usually reveals itself in athletic muscular subjects in early adult life, after the myocardium has become weakened by infection or unusual physical strain; and death commonly occurs in the prime of middle life, either abruptly from arterial rupture, or as a result of failing compensation in the presence of acute infections or their cardiac sequelae. Among these 200 cases, 148 (74 per cent) died before or during the fortieth year of life, so that, in spite of its rarity, this condition is one of great clinical and medico-legal importance. This fact is not sufficiently appreciated at the present time.

So-called "spontaneous" rupture of the heart or aorta (usually by way of a dissecting aneurysm in two stages) had taken place in 40 cases, in two of which the seat of rupture was in the right wall of the heart, in 33 in the ascending aorta, and in 5 at the seat of coarctation. The ascending aorta was dilated in practically all the cases of rupture in this situation and microscopic examination of its wall (made in 13 of the cases) usually showing medial degeneration, while a congenitally bicuspid valve was present in over 50 per cent of these 33 cases and in 22 per cent of the entire series. From these facts and the wide dissection of the tear a congenital weakness of the aortic media is suggested.

Infective endarteritis and mycotic aneurysm of the aorta took place in 14 cases, in 13 at the seat of coarctation, and in one immediately above the aortic valve; in the latter case death had taken place from rupture of the mycotic aneurysm, as also in 3 at the seat of coarctation. This makes a total of 44 cases of rupture of the aorta among the 200.

A serious neurological lesion was present in 26 cases, and in 20 of these it was cerebral hemorrhage. In 2 of these the hemorrhage was due to septic embolism, but in the other 18 it was "spontaneous" in origin, and in 5 of these the presence of a ruptured cerebral aneurysm was demonstrated at autopsy. In the remaining 13 cases the youth of the subjects, the symptoms of intermittent leakage, and the entire absence of any known cause, suggests that these also were due to rupture of a small congenital aneurysm, the result of a primary weakness of the wall of these arterioles of congenital origin.

THE RELATION OF INTRAVASCULAR PRESSURE TO ARTERIOSCLEROSIS. Eli Moschowitz, New York City.

Abstract. In the study of the etiology of arteriosclerosis one must take into consideration not merely a study of arteriosclerosis of the greater circulation but of the pulmonary circulation as well. Furthermore, a sclerosis comparable to the lesion of arteriosclerosis also occurs in the veins and especially the capillaries. Arteriosclerosis of the pulmonary circulation is independent of arteriosclerosis of the greater circulation, and only occurs when a condition causing increased pressure of the pulmonary circuit is present, such as mitral stenosis, emphysema, infiltrative lung lesions, open ductus Botalli or communications between the right and left heart. The pathology of the capillary lesions in pulmonary arteriosclerosis was discussed and the similarity of these to the glomerular lesions in hypertension of the greater circulation. The question of primary sclerosis of the pulmonary vessels — Does it occur? Decrescent arteriosclerosis of the greater circulation associated with long-continued normal intravascular pressure begins early in young adults. Decrescent arteriosclerosis of the pulmonary vessels occurs at a much later age, because the normal pulmonary pressure is one-sixth that in the aorta. The independence in incidence of arteriosclerosis between the greater and lesser circulations excludes obviously such causes as infections, metabolic products, etc.

The relation of experimental cholesterol deposits in the vessels to the problem of the etiology of arteriosclerosis was discussed, especially their similarity to the lipid deposits in the intima seen in infants on a high fat diet and in lipemia. These experimental lipid intimal infiltrations are probably not "arteriosclerosis." The subject of arteriosclerosis of the lower animals was reviewed, and the universality of arteriosclerosis in all peoples and in all ages. Arteriosclerosis occurs in those portions of the vessel that are fixed or have firm support. The only common factor in all arteriosclerosis is normal or increased intravascular pressure.

THE CIRCULATION OF BLOOD THROUGH THE SPLEEN PULP. Ward J. MacNeal, New York City.

Abstract. The splenic lobule consists of the malpighian corpuscle or follicle, its surrounding marginal zone and the irregularly radiating pulp cords surrounding this, together with the included vascular channels. Its periphery is marked by a zone of larger venous sinuses which serve adjacent lobules.

The intralobular or follicular arteriole is a terminal branch of the splenic artery without anastomosis with its fellows. From it there arise very abundant, extremely delicate vessels which form a capillary plexus within the follicle. These follicular capillaries communicate with each other. Their walls consist of a single continuous layer of delicate endothelium which retains the erythrocytes within the lumen but evidently permits the escape of fluid. In microscopic preparations most of these vessels are collapsed and appear as solid cords of endothelial cells. It appears probable that the passage of blood through them is intermittent in the living spleen. At the margin of the follicle these delicate capillaries terminate in the pulp at a considerable distance from any venous capillary. The endothelial cells of the wall become continuous with the reticular cells of the marginal zone and the lumen, if patent, opens into the intercellular spaces of the pulp. Sometimes a dilatation or rather poorly defined ampulla is recognizable at the termination.

Somewhat coarser branches of the follicular arteriole pass directly out of the follicle and curve about in the red pulp. These capillaries may give off several branches. They possess peculiarly thickened walls for part of their course, such a thickening constituting the ellipsoid sheath of Schweigger-Seidel. Some of the terminal twigs curve back toward the follicle as the centripetal capillaries of the marginal zone. The wall suddenly becomes reduced to a single sheet of endothelium and then the vessel opens out into the pulp spaces of the marginal zone, sometimes dividing and becoming distended before terminating.

Rather longer capillaries originate from the same trunk and extend along the pulp cords toward the periphery of the lobule, where they terminate in asymmetric ill-defined ampullae situated within the substance of the pulp cord. These ampullae open into the intercellular spaces of the pulp.

The intercellular spaces of the pulp open into the venous capillaries through the stomata of Mollier, which are present everywhere between the rod cells and the circular fibrils which form the walls of these capillaries. These vessels form a richly anastomosing plexus, running between the pulp cords from the marginal zone to the periphery of the lobule, where they pass over into coarser venous channels lined by a continuous sheet of endothelium and thence to the trabecular veins.

THE VENOUS DRAINAGE OF THE SPLEEN. W. L. Robinson, Toronto, Ont.

Abstract. The generally accepted conception of the structure of the venous sinuses of the spleen is that presented by Mollier (*Arch. f. Mikr. Anat.*, 1910-11, lxvi, 608). He described most minutely the variations in their structure and the general principles underlying them as seen in mammals and humans. One would gather from his article that the venous sinuses were a continuation of the arterial system, for he says, "The veins do not form an independent system but are merely canals formed in the pulp tissue." He points out that they are bounded either by the unchanged pulp tissue or in specially developed forms, *e. g.*, the human, with a protoplasmic syncytial lattice made up of longitudinal bands united by transverse bridges and supported by a reticulum; a perforated structure having stomata of various sizes and shapes. These apertures in the walls of the veins allow for the passage of the blood plasma and cellular elements from vein to pulp or from pulp to vein.

Using a Zeiss Binocular microscope with stereoscopic attachments, a study of the distended cat spleen reveals the fact that the venous system in the cat is

not continuous with the arterial system but is independent. The larger veins are enclosed wholly or in part by the trabeculae. The smaller branches are very short and made up of syncytial sheets of endothelial protoplasm which open out in a bell-shaped manner to communicate directly with the pulp spaces. The lining syncytium of the terminal veins blends almost imperceptibly with the pulp cells. There are a few rounded stomata in the walls of these terminal veins, but the main inlets are through the bell-shaped openings at their origins. The venous sinuses of the dog and human spleens are a little different from those of the cat in that their sinuses are much longer, more numerous, and have myriads of slit-like stomata in their walls. We have not as yet been able to demonstrate the beginnings of the individual venous branches in the dog and human spleens.

The findings in the cat spleen would seem to be further evidence of the independence of the arterial and venous capillary systems and positive proof of an open circulation for the spleen.

Discussion

(Dr. W. C. MacCarty, Rochester, Minn.) I am not clear in my own mind. He says the blood goes into the pulp from the arterioles, thence into the venous capillaries. I would like to know what the sinuses are. Maybe you made that clear but it was not clear to me.

(Dr. W. J. MacNeal, New York City.) It is true that if one has studied the perfused spleen it is possible to see in ordinary sections of the spleen more detail than before. I can say that is true in my own case. At the same time I do believe the routine perfusion of the spleen is something that is worth while and such technique as we have described does not interfere with the usual ordinary preparations. If you can get tissue two hours after death there is no reason why you cannot perfuse necropsy material. If you cut off a portion of the spleen from one end for ordinary fixation and smears and then clamp that end you can then go ahead and perfuse the remainder. The pulp of the spleen cannot be well fixed after four hours because the cells undergo lysis.

The discharge of blood from the blood vessels into the intercellular spaces of the pulp occurs most abundantly in the marginal zone around the follicles and I believe that the small capillaries within the follicle normally allow no red blood cells to pass through their walls but do allow the plasma to pass through. The concentrated corpuscles remain within the lumen to be discharged into the pulp spaces around the follicle. I regard this as a mechanism by which the fluid portion of the blood is brought into relation with the lymphocytes of the follicle where it may be acted upon chemically. The concentrated corpuscles are thrown into intercellular spaces of the marginal zone where they come into immediate relationship with the pulp cells and may be phagocyted or allowed to pass through if they are in good condition. Normally, the plasma and the corpuscles after passing this inspection join again in the pulp spaces and get back into the venous circulation through the stomata of Mollier.

As fast as we can work in the injection of particulate matter in suspension followed by perfusion fixation we find that the formed particles injected are always in the marginal zones. This material also escapes into the pulp cords but the amount here is much less. The marginal zone is evidently a most important part of the splenic lobule.

In reply to Dr. MacCarty's question as to the sinuses: I avoided the use of the term except perhaps through a slip of the tongue, because it may be misunderstood. In general the term sinuses designates the venous spaces and vessels of

the spleen which possess fenestrated walls composed of rod-shaped endothelial cells. The term does not apply to the intercellular spaces of the pulp.

(Dr. Robinson, closing.) Nothing more than to concur with Dr. MacNeal. We have made a number of India ink injections of the spleen. We found that if minimum amounts were injected intravenously it was filtered out by the ellipsoids. If larger quantities were injected it first appeared in the pulp proper about the malpighian corpuscles. I agree with Dr. MacNeal that the red blood cells pass on out through the end capillaries which are permeable, into the pulp spaces.

RETICULUM IN THE LUNG IN POST-INFLUENZAL ORGANIZATION. G. R. Callender, Washington, D. C.

Abstract. The organizing processes in the exudate in alveoli, air sacs, atria and bronchioles in the pneumonic processes following the 1918 pandemic of influenza were studied to determine the sequence of events leading to the final organization into fibrous connective tissue. The steps in order were found to be as follows:

1. Increase in large mononuclear cells (histiocytes) in the walls of atria and alveoli and in the exudate. The latter was usually granular with little fibrin, rarely fibrinous, occasionally purulent.

2. Extension of reticulum fibers from the reticulum of the walls into the exudate.

3. Formation of collagen by fibroblasts in the exudate.

4. Disappearance of reticulum as the collagen increased but without evidence that reticulum was transformed into collagen.

Delicate fibers of both collagen and reticulum were present in the same area in the exudate. Because of this finding collagen fibers are considered to differ chemically from reticulum fibers.

Discussion

(Dr. N. C. Foot, Cincinnati.) We shall never get the answer as to the difference between reticulum and collagen by means of staining methods alone; we can never decide as to whether they are chemically different until we approach the question from the chemical standpoint. I believe that we have exhausted the resources and utility of staining methods in this connection; we can get just so far and then we are up against a barrier. For that reason I have been tackling the subject from the standpoint of chemical differences, or chemistry. If you take a small piece of spleen and boil it in water for four hours, you will find that you are unable to stain it with acid fuchsin, the staining properties of the collagen are lost. Furthermore, if you evaporate this aqueous extract down to a syrup, you can recover the collagen in the extract; in other words, what we call "collagen" (from the microscopical standpoint) is apparently a material soluble in hot water. It is not gelatinous. On the other hand, if you boil spleen in water the reticulum does not dissolve. If you boil it in alcohol the reticulum will be more or less affected and dispersed through the tissue. Digestion with pancreatin destroys all of the cellular material in a spleen, leaving the connective tissue undigested; if you dissolve this in weak alkali and neutralize the solution, you will get a precipitate in which you can (with the silver carbonate-fuchsin method) stain material that looks very much like reticulum. There will be typical fibrils in this material. On the other hand, if you wash this precipitate, there will be nothing much that will stain with acid fuchsin. We are appar-

ently dealing with two substances that are closely related and I believe that, in the case of reticulum, collagen may be associated with some other substance that gives this tissue its characteristic argyrophilic tendencies.

If you use the silver carbonate method on formalin-fixed material, you may or may not get a reticulum impregnation, but if you will first subject this tissue to the Mallory method of oxidation and reduction (potassium permanganate followed by oxalic acid) you are almost certain to get 100 per cent impregnation of the reticulum. If, on the other hand, you treat such tissues with reducing agents, such as pyrogallol or sodium sulphite, you can entirely inhibit the impregnation of reticulum. All this has no effect upon the collagen whatsoever. So it seems to me that we are gradually accumulating evidence that there is a chemical difference between these substances.

Dr. Mallory brought out, last year, a statement that this is merely a mechanical difference, the compact fibrils (which are loosely arranged) responding to silver and the more diffuse fibrils that are felted together in fibrous masses not being permeable to the silver solution and therefore not impregnating. I think that, if this were true, we should get a silver mirror over the surface of the sections after silver treatment, which is not the case.

I submit these points for your consideration.

(Dr. E. B. Krumbhaar, Philadelphia.) I ask a question again for information. Did not Dr. Mallory satisfactorily show last year as regards the histogenesis, that both reticulum and collagen could come from fibroblasts?

(Dr. Callender, closing.) I want to thank Dr. Foot for his discussion. In regard to Dr. Krumbhaar's question. It is my understanding that what Dr. Mallory said last year was that collagen and reticulum were one and the same and it depended upon the size of the fiber whether it would take up the reticulum or collagen stain. Therefore, it was implied that they both arise from the same cell, namely fibroblasts.

A reticulum cell is indistinguishable to me from a young fibroblast, but there will be reticulum in one place and in another collagen.

METADYSENTERY, WITH REMARKS ON CERTAIN GROUPS OF INTESTINAL BACTERIA. Aldo Castellani (by invitation), New Orleans, La.

Abstract. Some years ago I introduced the following classification of bacterial dysenteries:

1. Bacterial dysentery *sensu stricto*, due to the Shiga-Kruse bacillus (does not ferment lactose, does not ferment mannitol).
2. Paradyentery, due to Flexner, and Flexner-like bacilli including Hiss-Russell (does not ferment lactose, ferments mannitol).
3. Metadyentery, due to the "metadyentery bacilli." The "metadyentery bacilli" are organisms which like the true dysentery bacilli (Shiga, Flexner and Flexner-like or dysentery-paradyentery organisms) do not produce gas in any sugar, but in contrast to the true dysentery bacilli they produce acidity to litmus in lactose peptone water and clot milk, or produce acidity in lactose without clotting in milk or clot milk without producing distinct acidity to litmus in lactose peptone water.

These bacilli were grouped by Chalmers and myself into two genera dysenteroides and lankoides. The organisms of the dysenteroides group do not clot milk, those of the lankoides group clot milk. In practice, however, it is of advantage to combine the two genera into one (*dysenteroides sensulato*) with the general characters I have mentioned.

Principal types of the metadysentery bacilli

The following bacilli may be considered to belong to the metadysentery group:

1. *Bacillus pyogenes foetidus* Passet 1902. This and similar organisms although belonging culturally to the metadysentery group do not seem to cause dysentery or any other clinical type of colitis. They were found in abscesses.

2. The bacilli found by me in dysentery and certain peculiar types of colitis febrile and afebrile, which I described with the names *B. ceylonensis A*; *B. ceylonensis B*; *B. metadysentericus*. In addition *B. gintlottensis* and *B. madampensis*.

3. Some strains discovered by Duval in his important investigation on the etiology of infantile diarrhoea seem to me to belong to the metadysentery group rather than to the paradysentery group or Flexner group.

4. Certain strains found in England in cases of infantile diarrhoea by Nabarro who, however, until recently identified them with *B. coli anaerogenes*.

5. Certain strains found recently by Sonne in cases of dysentery unless these strains be identical with *B. ceylonensis A*; *B. ceylonensis B*; and *B. metadysentericus*.

The pathogenic metadysentery bacillary may be separated into two principal types. (1) *Bacillus ceylonensis A* type (including *B. metadysentericus*); (2) *Bacillus ceylonensis B* type. In the first type acidity in lactose peptone water is produced very slowly and clotting of milk may be absent. Indol is usually negative. In the second type acidity in lactose is produced rapidly and milk is clotted also fairly rapidly. Indol is usually positive.

There are several different types of metadysentery. In this paper I have called attention to a chronic type which is not of rare occurrence. In this type true dysenteric symptoms, *viz.*, blood and pus in the stool are often lacking. The patient feels tired, nervous, depressed, has no appetite, complains of vague abdominal discomfort, dyspepsia, flatulency, with occasional attacks of diarrhoea often followed by long periods of constipation. A similar clinical syndrome may be caused by *Entamoeba histolytica* but rarely by bacilli of the Shiga and Flexner type.

I shall be pleased to supply workers interested in the subject with cultures of the strains of metadysentery bacilli which I have in my possession.

THE BIOLOGICAL ACTION OF RADIANT ENERGY. I. ULTRAVIOLET. Eugene R. Whitmore, Washington, D. C.

(Abstract not received.)

STUDIES ON LIPOCHROMES. Charles L. Connor, Boston, Mass.

Abstract. This report deals with several aspects of the question, the first of which had to do with the reaction of animals to carotin. This, the principal lipochrome found in the animal body, was extracted from carrots and purified. Upon injection intraperitoneally into guinea pigs granulomatous lesions are formed similar to familiar foreign body reactions. It does not appear in the blood or urine of guinea pigs after injection or ingestion of relatively large amounts. It produced no effect in a rabbit when injected intravenously.

By the use of fat stains on frozen sections of carrots, adrenal gland, corpus luteum, and chicken skin, and on tissues removed from animals after injection of cholesterol and other lipoids, and by the use of other reagents which might be expected to distinguish lipochromes, an effort was made to differentiate carotin, xanthophyll, cholesterol, and other lipoids from one another. It was found that

lipochromes do not stain characteristically with fat stains; that Nile blue sulfate does not differentiate cholesterol from other lipoids except neutral fat; but that, by its double refractiveness, lipochrome is most often associated with cholesterol or an ester of cholesterol. Lipochrome is easily bleached with FeCl_3 , is soluble in CHCl_3 after dehydration with acetone, and crystallizes after treatment with alcoholic KOH and formalin. It cannot be seen normally as a granular substance in any tissue which contains it except the outer layers of the skin, because of its fine dispersion in its fatty solvent.

Lipochrome was found by chemical or histologic methods to be present in the skin, fat, liver, spleen, adrenal cortex, and corpus luteum. Ten methods, chemical and histologic, including those found to be useful in identifying carotin, and the dopa reaction, silver nitrate, basic fuchsin, and iron stains, were employed to differentiate the other common pigments. Hemofuscin and hemosiderin were found to be present together in the heart, intestinal muscle, seminal vesicles, testicles, prostate, and in old hemorrhagic foci. These two pigments are increased in brown atrophy of the heart and liver. Melanin seems to be confined to nervous tissue or ectodermal cells. Two pigments, therefore, increase in amount in advancing age, namely, lipochrome, which is exogenous and comparatively unimportant, and hemofuscin with an iron-containing pigment, which may be derived from muscle hemoglobin.

ABERRANT THYROID GLANDS. Lawrence W. Smith and (by invitation) John V. Leech, Boston, Mass.

Abstract. Four cases of lateral aberrant thyroid tumors are presented with the clinical and pathological findings. Their probable etiology from the so-called fifth pharyngeal pouch is discussed. The possible relationship of the papilliferous tumors of the thyroid gland itself to these same cell rests, after migration, is raised.

SARCOMAS OF THE LIVER. Ernest Scott and (by invitation) Harry L. Reinhart, Columbus, Ohio.

Abstract. The paper is concerned with the presentation of an unusual case of multiple primary tumors of the liver, arising in the reticulo-endothelial system of that organ.

The tumor is of a polymorphous character, and the microscopic sections reveal areas in which there is a marked proliferation of reticulo-endothelial cells, with destruction of liver parenchyma, or formation of an endothelial syncytium. Other areas are sarcomatous with diffuse hemorrhages scattered throughout the substance.

TUMORS AND MALFORMATIONS OF THE BLOOD VESSELS OF THE CENTRAL NERVOUS SYSTEM. Percival Bailey (by invitation), Boston, Mass.

Abstract. Tumors of the cerebral blood vessels may be divided essentially into vascular malformations (telangiectases, angioma venosum, angioma arteriale) and hemangioblastomas. The former are composed of vessels (capillary, venous or arterial) separated by neuroglia. The latter are true neoplasms composed of angioblasts which may form either capillary spaces, solid masses of endothelial cells or cavernous spaces filled with blood or serum. No neuroglia is found within these tumors but an abundant reticulum is present. The hemangioblastomas are most common in the cerebellum and are often cystic. They are often associated with angiomatosis of the retina and sometimes with cystic pancreas,

cystic kidneys, hypernephromata, etc., in a pathological complex now known as Lindau's disease.

Examples of the different types of these tumors will be described with the aid of lantern slides.

A CONTRIBUTION TO THE PATHOLOGY AND ETIOLOGY OF MEXICAN TYPHUS.
H. Mooser (by invitation), Mexico City, Mexico.

Abstract. Male guinea pigs inoculated with Mexican typhus show swelling and inflammation of the scrotum. Histological examination reveals that the primary lesion takes place within the endothelial lining of the tunica vaginalis. Stained smears from the proliferated tunica show swollen endothelial cells containing a minute diplo-bacillus which multiplies so abundantly that the cells finally rupture. This liberation of the microorganism is followed by the local reaction and fever. The infected cells exhibit a striking resemblance to the stomach cells of lice containing *Rickettsia prowazeki*. The tunica is highly infectious as is evidenced by inoculation into guinea pigs and the accidental infection of a laboratory worker with typhus.

Discussion

(Dr. Lillie, Washington.) I have had an opportunity in the past year to compare the histological tissues of typhus from Dr. Wolbach's laboratory and from the one isolated by Dr. Maxcy. I think we can fully confirm Dr. Connor's statements of the entire absence of the scrotal lesions, whereas we find the same thing that Dr. Mooser described in Mexican typhus. I had occasion to look at a section of Dr. Mooser's this morning and some of my own and I would hate to tell which is which. I have one photographic negative which I took last evening which shows the similarity of what we have to what Dr. Mooser described.

In the brains of the cases which we were studying we were able to see typical nodes in about one-half the guinea pigs which I think is intermediate between what Dr. Mooser finds and the European strain. I might also say in spotted fever I have not found them in the few animals I have examined.

(Dr. Mooser.) Dr. Wolbach was in Mexico in 1919 and published his first report on Mexican typhus and he did not find any difference. I did not see any difference in Mexican typhus and European typhus except that the rash in Mexican typhus was paler than European typhus, beginning like measles and becoming hemorrhagic later. The brain lesions are much more rare in Mexican typhus in human beings than in guinea pigs and we were always able to find them in guinea pigs. I might observe that the scrotal swelling was probably responsible for the rarity of the lesions. The female will show many lesions in the gray matter of the brain. The male shows very few lesions. The testicle protects the brain from being involved. The lesion in the vascular endothelium is always most pronounced.

(Dr. C. L. Connor, Boston.) The lesion which has been described and the picture which I have seen is extremely like that of the lesion of Rocky Mountain fever. Ricketts, in 1908, called attention to the similarity between typhus fever and Rocky Mountain spotted fever as far as clinical lesions and the external lesions produced in humans are concerned. No one has ever described before such a lesion in the guinea pig in true typhus fever or typhus fever in Europe, and in the strain which has been carried on in the Harvard Medical School no such lesion has ever appeared, although these animals have been injected intraperitoneally. I think the route of injection has nothing to do with it. On the

other hand, these lesions occur in Rocky Mountain spotted fever although they are injected subcutaneously or in some other way. It has been demonstrated that whereas spotted fever is carried by a particular tick in the spotted fever region, it may also be carried by any other tick; so that the fact that typhus and Mexican typhus, which seemed to be the same until now, may not be carried by the same thing is not surprising. It is most important to find out what the carrier is; whether it may not be a tick and whether it is not more closely related to Rocky Mountain fever than European typhus.

(Dr. Maxcy, Washington.) I think Dr. Mooser has made a very important contribution to our knowledge of typhus. If Rickettsia-like bodies can be demonstrated consistently and convincingly in guinea pigs experimentally infected with typhus virus, then the strongest argument against the etiologic relationship of Rickettsia to typhus will have broken down.

We are more interested, however, in the possibility that the pathologic lesion which Dr. Mooser describes may be used to differentiate North American typhus from Old World typhus. Dr. M. H. Neill of the U. S. Public Health Service first described swelling and redness of the scrotum of guinea pigs inoculated with a strain of typhus isolated on the Mexican border. Dr. Mooser has confirmed and extended this observation so that its specific character seems established. I have found the same lesion present in guinea pigs inoculated with the endemic typhus of the southern United States, which by the way is not conveyed by lice. In other respects these guinea pigs reacted as do guinea pigs inoculated with Old World typhus. Dr. Lillie has made a detailed study of the microscopic pathology and in the main his observations confirm Mooser. The pathology of guinea pigs inoculated with Mexican typhus and with the endemic typhus of the United States appears to be similar, and to present certain characteristics which are different from those usually manifested in guinea pigs inoculated with Old World typhus.

(Dr. A. Plaut, New York City.) Can one really compare the nodules which were shown outside the infarcted area with nodules in the tissue? I never saw nodules in typhus in any organ, including the central nervous system, which did not chiefly consist of histiocytes, together with some plasma cells, occasional leucocytes, etc. These nodules on serial sections always were found connected with a small blood vessel. I wonder if these loosely lying masses which were shown can be compared with the typhus nodule.

(Dr. Allen, Charlotte, N. C.) In dealing with endemic typhus in the south it is simple to convey it directly to guinea pigs. We have not been able to demonstrate in the brain the pathology described by Dr. Wolbach but have noticed constantly the orchitis demonstrated this morning. Neither lice nor ticks have anything to do with our endemic typhus.

THE EARLY DIAGNOSIS OF MALIGNANCY. W. C. MacCarty, Rochester, Minn.

(Abstract not received.)

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